Check In 1

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2025-09-27

```
#READING IN
#all necessary libraries
library(dplyr)
##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##
      filter, lag
## The following objects are masked from 'package:base':
##
      intersect, setdiff, setequal, union
##
library(ggpubr)
## Loading required package: ggplot2
library(ggplot2)
library(gridExtra)
##
## Attaching package: 'gridExtra'
## The following object is masked from 'package:dplyr':
##
##
      combine
library(grid)
library(ComplexHeatmap)
## ComplexHeatmap version 2.24.1
## Bioconductor page: http://bioconductor.org/packages/ComplexHeatmap/
## Github page: https://github.com/jokergoo/ComplexHeatmap
## Documentation: http://jokergoo.github.io/ComplexHeatmap-reference
```

```
##
## If you use it in published research, please cite either one:
## - Gu, Z. Complex Heatmap Visualization. iMeta 2022.
## - Gu, Z. Complex heatmaps reveal patterns and correlations in multidimensional
      genomic data. Bioinformatics 2016.
##
## The new InteractiveComplexHeatmap package can directly export static
## complex heatmaps into an interactive Shiny app with zero effort. Have a try!
## This message can be suppressed by:
    suppressPackageStartupMessages(library(ComplexHeatmap))
library(circlize)
## ===============
## circlize version 0.4.16
## CRAN page: https://cran.r-project.org/package=circlize
## Github page: https://github.com/jokergoo/circlize
## Documentation: https://jokergoo.github.io/circlize_book/book/
## If you use it in published research, please cite:
## Gu, Z. circlize implements and enhances circular visualization
    in R. Bioinformatics 2014.
##
## This message can be suppressed by:
    suppressPackageStartupMessages(library(circlize))
## =============
library(ggbeeswarm)
library(ggdist)
#reading in the counts
my_data <- read.csv("C:/Users/savan/Downloads/counts.csv", header = TRUE, row.names = 1, )</pre>
#this is just small portion of the data for me to view (head) is too large
my_data[1:12, 1:2]
                     TCGA.GM.A2DL.01A.11R.A18M.07 TCGA.AC.A2QI.01A.12R.A19W.07
## ENSG0000000003.15
                                            1262
                                                                        2922
## ENSG0000000005.6
                                             120
                                                                           4
## ENSG0000000419.13
                                            1535
                                                                        1779
## ENSG0000000457.14
                                             885
                                                                        2574
## ENSG0000000460.17
                                             328
                                                                         586
## ENSG0000000938.13
                                             604
                                                                         625
## ENSG0000000971.16
                                            2737
                                                                        3771
## ENSG0000001036.14
                                            2370
                                                                        2517
## ENSG0000001084.13
                                            1139
                                                                        1688
## ENSG0000001167.14
                                            2543
                                                                        2153
## ENSG0000001460.18
                                                                         845
                                             559
## ENSG0000001461.17
                                            2204
                                                                        2653
```

```
#READING IN
#reading in the metadata
my_metadata <- read.csv("C:/Users/savan/Downloads/meta_data.csv", header = TRUE,)</pre>
#SUMMARY STATS
#I chose the gene on the 11th line and computed the summary statistics
#I had to make the data a numeric vector since it was a row
my_gene <- as.numeric(my_data[11,])</pre>
#summary
summary_mygene <- summary(my_gene)</pre>
#standard deviation
sd_mygene <- sd(my_gene)</pre>
#adding standard dev and summary into a vector
summary_mygene <- c(summary_mygene, "Standard Deviation" = sd(my_gene))</pre>
summary_mygene
##
                 Min.
                                1st Qu.
                                                     Median
                                                                           Mean
           105.0000
                              474.5000
                                                  652.0000
                                                                       699.8773
##
             3rd Qu.
                                    Max. Standard Deviation
                                                  318.0273
##
            866.5000
                             2620.0000
#making a data fram of summary + sd
summary_df <- data.frame(summary_mygene)</pre>
#labeling the column of values
colnames(summary_df) <- "Value"</pre>
#adding my own row name
summary_df <- cbind(`Summary_Statistics` = rownames(summary_df), summary_df)</pre>
#qetting rid of default row names
rownames(summary_df ) <- NULL</pre>
#viewing the summary df
summary df
    Summary Statistics
                           Value
## 1
                   Min. 105.0000
## 2
              1st Qu. 474.5000
               Median 652.0000
## 3
                   Mean 699.8773
## 4
## 5
               3rd Qu. 866.5000
## 6
                  Max. 2620.0000
## 7 Standard Deviation 318.0273
```

```
#open PNG device
png("dataframe_image.png", width = 800, height = 400)
#converting df to table and loading it
#I used https://cran.r-project.org/web/packages/gridExtra/vignettes/tableGrob.html to figure this out
#Adding a title
grid.text("Summary Statistics for ENSG00000001460.18\n(RNA-Seq Counts)", y = 0.78, gp = gpar(fontsize =
#drawing the tabe
grid.table(summary_df, rows = NULL)
#close device
dev.off()
## pdf
## 2
#SUMMARY STATS
#I chose the gene on the 12th line and computed the summary statistics
#I had to make the data a numeric vector since it was a row
my_gene2 <- as.numeric(my_data[12,])</pre>
#summary
summary_mygene2 <- summary(my_gene2)</pre>
sd_mygene2 <- sd(my_gene2)</pre>
#vector of all stats
summary_mygene2 <- c(summary_mygene2, "Standard Deviation" = sd(my_gene2))</pre>
summary_mygene2
##
                 Min.
                                 1st Qu.
                                                      Median
                                                                             Mean
                                                                          3079.09
##
               232.00
                                 1658.00
                                                      2516.00
##
              3rd Qu.
                                     Max. Standard Deviation
                               15355.00
##
              3934.00
                                                     2120.10
#df of stats
summary_df2 <- data.frame(summary_mygene2)</pre>
#making my own column/row names again
colnames(summary_df2) <- "Value"</pre>
summary_df2 <- cbind(`Summary Statistics` = rownames(summary_df2), summary_df2)</pre>
rownames(summary_df2 ) <- NULL</pre>
#viewing
summary_df2
    Summary Statistics
                           Value
                          232.00
## 1
                   Min.
```

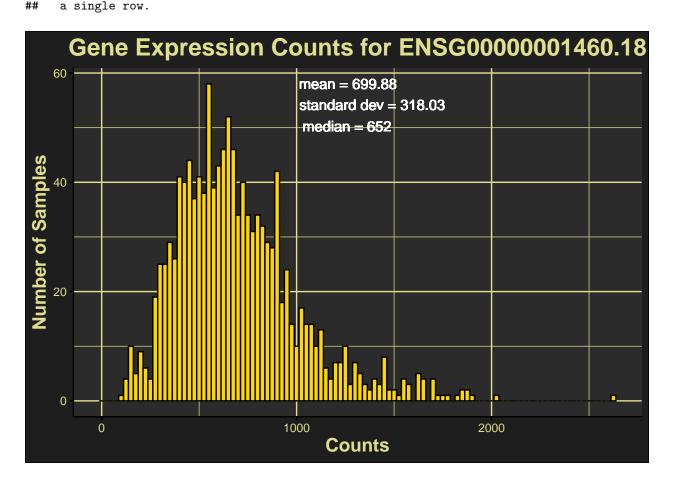
```
## 2
                1st Qu. 1658.00
## 3
                Median 2516.00
                   Mean 3079.09
## 4
## 5
                3rd Qu. 3934.00
## 6
                   Max. 15355.00
## 7 Standard Deviation 2120.10
#open device
png("dataframe_image2.png", width = 800, height = 400)
#drawing and customizing the table
grid.text("Summary Statistics for ENSG00000001461.17\n(RNA-Seq Counts)", y = 0.78, gp = gpar(fontsize =
grid.table(summary_df2, rows = NULL)
#close device
dev.off()
## pdf
##
   2
#HISTOGRAM FOR GENE 1
#turned my gene data into a data frame
my_gene_df <- data.frame(my_gene)</pre>
#making histogram. I am using various elements to change the color because it is fun!
#My resource for color customization is https://www.sthda.com/english/wiki/ggplot2-themes-and-backgroun
plot<- ggplot(my_gene_df, aes(x = my_gene)) +</pre>
  geom_histogram(binwidth = 25, fill = "gold", color = "black") +
 labs(title = "Gene Expression Counts for ENSG00000001460.18",
       x = "Counts".
       y = "Number of Samples") + theme_classic() +
  theme(
  plot.background = element_rect(fill = "#1e1e1e", color = "black"),
  panel.background = element_rect(fill = "#2b2b2b", color = NA),
  plot.title = element_text(face = "bold", size = 18, hjust = 0.5, color = "#e0e090"),
                                                                                           # gold
 axis.title = element_text(face = "bold", size = 14, color = "#e0e090"),
  axis.text = element_text(color = "#e0e090"),
  panel.grid.major = element_line(color = "#e0e090"),
  panel.grid.minor = element_line(color = "#e0e090"), axis.line.x = element_line(color = "black"))
#taking the mean of the counts
mean1 <- round(mean(my_gene),2)</pre>
#taking the sd of the counts
sd1 <- round(sd(my_gene), 2)</pre>
#taking the median of the counts
median1 <- round(median(my_gene),2)</pre>
\#I added some important statistical annotations
#looked up and figured out geom_text from https://stackoverflow.com/questions/53799878/how-to-format-gg
```

```
## i Please consider using 'annotate()' or provide this layer with data containing
## a single row.

## Warning in geom_text(aes(x = 3.6, y = Inf), label = paste0("standard dev = ", : All aesthetics have
## i Please consider using 'annotate()' or provide this layer with data containing
## a single row.

## Warning in geom_text(aes(x = 3.6, y = Inf), label = paste0("median = ", : All aesthetics have length
## i Please consider using 'annotate()' or provide this layer with data containing
```

Warning in geom_text(aes(x = 3.6, y = Inf), label = paste0("mean = ", mean1), : All aesthetics have

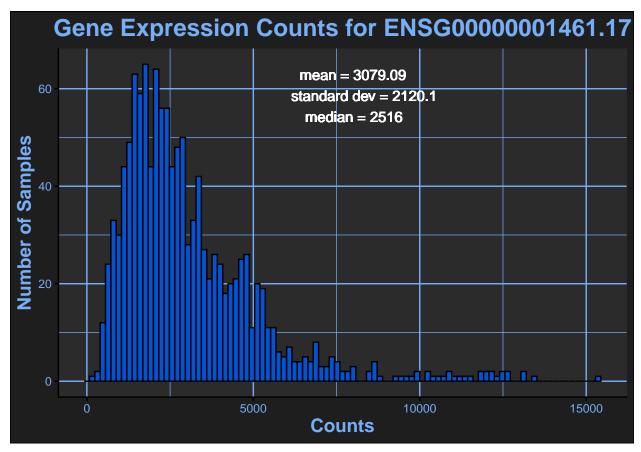


```
#making histogram. I am using various elements to change the color because it is fun!
plot2 <- ggplot(my_gene_df2, aes(x = my_gene2)) +</pre>
  geom_histogram(binwidth = 160, fill = "#0453d1", color = "black") +
  labs(title = "Gene Expression Counts for ENSG00000001461.17",
       x = "Counts",
       y = "Number of Samples") + theme_classic() +
  plot.background = element_rect(fill = "#1e1e1e", color = "black"),
   panel.background = element_rect(fill = "#2b2b2b", color = NA),
   plot.title = element_text(face = "bold", size = 18, hjust = 0.5, color = "#78aef5"),
  axis.title = element_text(face = "bold", size = 14, color = "#78aef5"),
  axis.text = element_text(color = "#78aef5"),
  panel.grid.major = element_line(color = "#78aef5"),
  panel.grid.minor = element_line(color = "#78aef5"),
  axis.line.x = element_line(color = "black"))
#taking the mean of the counts
mean2 <- round(mean(my_gene2),2)</pre>
#taking the sd of the counts
sd2 <- round(sd(my_gene2), 2)</pre>
#taking the median of the counts
median2 <- round(median(my_gene2),2)</pre>
#I added some important statistical annotations
histogram2 <- plot2 +
            geom_text(aes(x = 3.6, y = Inf), label = paste0("mean = ", mean2),
                      vjust = 3,hjust = -2, size = 4, color = "white", inherit.aes =
                        FALSE) +
            geom_text(aes(x = 3.6, y = Inf), label = paste0("standard dev = ", sd2),
                      vjust = 5,hjust = -1.4, size = 4,color = "white", inherit.aes =
                        FALSE ) +
            geom_text(aes(x = 3.6, y = Inf), label = paste0("median = ", median2),
                      vjust = 7,hjust = -2.24, size = 4,color = "white", inherit.aes =
#printing entire histogram
histogram2
## Warning in geom_text(aes(x = 3.6, y = Inf), label = paste0("mean = ", mean2), : All aesthetics have
## i Please consider using 'annotate()' or provide this layer with data containing
    a single row.
## Warning in geom_text(aes(x = 3.6, y = Inf), label = paste0("standard dev = ", : All aesthetics have
## i Please consider using 'annotate()' or provide this layer with data containing
   a single row.
## Warning in geom_text(aes(x = 3.6, y = Inf), label = paste0("median = ", : All aesthetics have length
```

#turned my gene data for second gene into a data frame

my_gene_2 <- as.numeric(my_data[12,])
my gene df2 <- data.frame(my gene2)</pre>

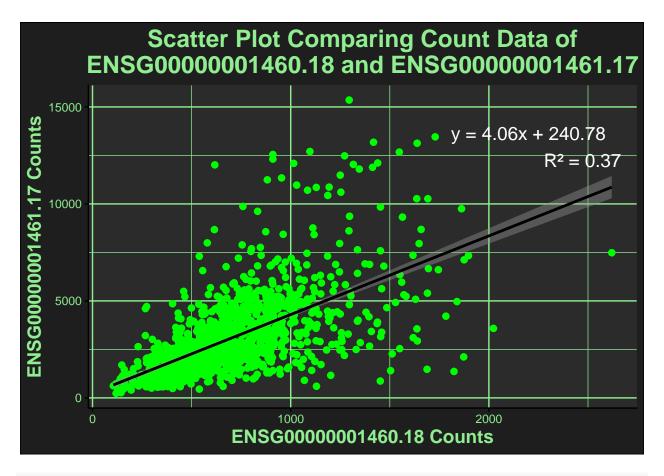
i Please consider using 'annotate()' or provide this layer with data containing
a single row.



```
#SCATTER PLOT
#Taking second gene and making it a numeric vector
my_gene_2 <- as.numeric(my_data[12,])</pre>
#making a df with both genes and ensuring the columns line up
scatter_df <- data.frame(my_gene, my_gene_2)</pre>
#beginning to add regresion line
model <- lm(my_gene_2 ~ my_gene, data = scatter_df)</pre>
#finding the R squared value. I used https://stackoverflow.com/questions/23519224/extract-r-square-value
r_squared <- summary(model)$r.squared</pre>
r_label <- paste0("R2 = ", round(r_squared, 2))</pre>
#extract coefficients
coefficients <- coef(model)</pre>
intercept <- coefficients[1]</pre>
slope <- coefficients[2]</pre>
#create equation label text
eq_label <- paste0("y = ",
```

round(slope, 2), "x + ",

'geom_smooth()' using formula = 'y ~ x'



#COMBINING COUNT DATA AND METADATA

 $\#In\ order\ to\ ensure\ that\ the\ covariate\ and\ counts\ lined\ up\ in\ columns\ I\ decided\ to\ transpose\ my\ count\ d\ counts_transposed\ <-\ as.data.frame(t(my_data))$

#changing the periods for dashes for easy matchup

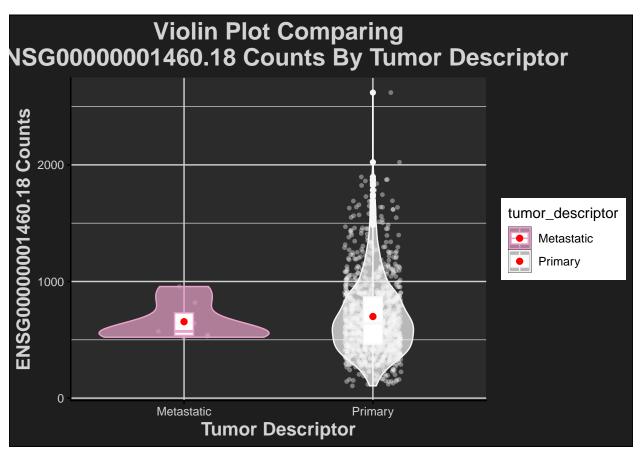
```
rownames(counts_transposed) <- gsub("\\.", "-", rownames(counts_transposed))</pre>
#giving the samples a header for easy merging.
counts_transposed$barcode <- rownames(counts_transposed)</pre>
#merging both by the sample ID (barcode) in case they are in differet orders
main_df <- merge(counts_transposed, my_metadata, by = "barcode")</pre>
#checking that meta_data is at the end of my main data frame
main df[1:12, 60740:60758]
##
      paper_days_to_death paper_days_to_last_followup
## 1
                         NA
                                                     4047
## 2
                                                     4005
                         NA
## 3
                         NA
                                                     1474
## 4
                         NA
                                                     1448
## 5
                         NA
                                                      348
## 6
                         NA
                                                     1477
## 7
                         NA
                                                     1471
## 8
                         NA
                                                      303
## 9
                         NA
                                                      259
## 10
                         NA
                                                      437
## 11
                         NA
                                                     1321
## 12
                         NA
                                                     1463
##
      paper_age_at_initial_pathologic_diagnosis paper_pathologic_stage
## 1
## 2
                                                50
                                                                   Stage_II
## 3
                                                62
                                                                   Stage_II
## 4
                                                52
                                                                    Stage_I
## 5
                                                50
                                                                  Stage_III
## 6
                                                42
                                                                   Stage_II
## 7
                                                63
                                                                   Stage_IV
## 8
                                                52
                                                                   Stage_II
## 9
                                                70
                                                                    Stage I
## 10
                                                                   Stage_II
                                                59
## 11
                                                56
                                                                    Stage_I
## 12
                                                54
                                                                   Stage_II
      paper_Tumor_Grade paper_BRCA_Pathology paper_BRCA_Subtype_PAM50
##
## 1
                                           <NA>
                      NA
                                                                      LumA
## 2
                                           <NA>
                                                                      Her2
                      NA
## 3
                      NA
                                           <NA>
                                                                      LumB
## 4
                      NA
                                           <NA>
                                                                      LumA
## 5
                      NA
                                           <NA>
                                                                      LumA
## 6
                                           <NA>
                                                                      LumA
                      NA
## 7
                      NA
                                           <NA>
                                                                      LumA
## 8
                      NA
                                           <NA>
                                                                      LumB
## 9
                      NA
                                          Other
                                                                    Normal
## 10
                      NA
                                            IDC
                                                                      LumA
## 11
                      NA
                                            ILC
                                                                      LumA
## 12
                                                                      LumA
##
      paper_MSI_status paper_HPV_Status paper_tobacco_smoking_history
## 1
                                        NA
                     NA
                                                                        NA
## 2
                                        NA
                                                                        NA
```

```
## 3
                      NA
                                         NA
                                                                           NA
## 4
                      NΑ
                                         NΑ
                                                                           NΑ
## 5
                      NA
                                         NA
                                                                           NA
## 6
                                                                           NA
                      NA
                                         NA
## 7
                      NA
                                         NA
                                                                           NA
## 8
                      NA
                                         NA
                                                                           NA
## 9
                      NA
                                         NA
                                                                           NA
## 10
                      NA
                                         NA
                                                                           NA
## 11
                      NA
                                         NA
                                                                           NA
## 12
                      NA
                                         NA
                                                                           NA
      paper_CNV.Clusters paper_Mutation.Clusters paper_DNA.Methylation.Clusters
## 1
                        C6
                                                   C7
## 2
                        C6
                                                   C9
                                                                                       C2
## 3
                        C6
                                                   C4
                                                                                      C2
## 4
                        C1
                                                   C5
                                                                                       C2
## 5
                        C6
                                                    C4
                                                                                      C1
## 6
                        C1
                                                   C9
                                                                                      C1
## 7
                      <NA>
                                                   C9
                                                                                      C1
## 8
                        C6
                                                   C6
                                                                                      C2
## 9
                        C2
                                                    C4
                                                                                      C3
## 10
                        C4
                                                   C4
                                                                                      C1
## 11
                        C1
                                                    C4
                                                                                      C1
## 12
                        C5
                                                   C4
                                                                                      C1
##
      paper_mRNA.Clusters paper_miRNA.Clusters paper_lncRNA.Clusters
## 1
                         C1
                                                 СЗ
                                                                        <NA>
## 2
                         C2
                                                 СЗ
                                                                        <NA>
## 3
                         C2
                                                 C2
                                                                        <NA>
## 4
                          C2
                                                 C2
                                                                        <NA>
## 5
                         C2
                                                 C2
                                                                        <NA>
## 6
                         C2
                                                 C2
                                                                        <NA>
## 7
                         C2
                                               <NA>
                                                                        <NA>
## 8
                          C2
                                                 C3
                                                                        <NA>
## 9
                          C4
                                                 C4
                                                                        <NA>
                                                 СЗ
## 10
                         C1
                                                                        <NA>
                                                 СЗ
                         C1
## 11
                                                                        <NA>
                                                 СЗ
## 12
                         C1
##
      paper_Protein.Clusters paper_PARADIGM.Clusters paper_Pan.Gyn.Clusters
## 1
                           <NA>
                                                        C5
                                                                                <NA>
## 2
                             C2
                                                        C4
                                                                                  C4
## 3
                           <NA>
                                                        C4
                                                                                <NA>
## 4
                             C2
                                                        C6
                                                                                  C4
## 5
                             C2
                                                                                  C1
                                                        C6
## 6
                           <NA>
                                                        C6
                                                                                <NA>
## 7
                             C2
                                                                                  C1
                                                      <NA>
## 8
                             C2
                                                        C8
                                                                                  C5
## 9
                           <NA>
                                                        C6
                                                                                <NA>
## 10
                           <NA>
                                                        C6
                                                                                <NA>
## 11
                           <NA>
                                                        C5
                                                                                <NA>
                                                        C6
## 12
                             C1
                                                                                  C1
```

#VIOLIN PLOT

#Taking out non applicable values because I just want to focus on primary and metastatic
main_df <- main_df[main_df\$tumor_descriptor != "Not Applicable",]</pre>

```
#putting together the violin plot for my gene and tumor descriptor
ggplot(main_df, aes(x = tumor_descriptor, y = ENSG00000001460.18, fill = tumor_descriptor, color = tumor
geom_violin(alpha = 0.7) + geom_jitter(width = 0.15, size = 1, alpha = 0.4) + geom_boxplot(width=0.1, stat_summary(fun = mean, geom = "point", shape = 20, size = 3, color = "red") +
labs(x = "Tumor Descriptor", y = "ENSG00000001460.18 Counts", title = "Violin Plot Comparing\nENSG000
plot.background = element_rect(fill = "#1e1e1e", color = "black"),
panel.background = element_text(fail = "#2b2b2b", color = NA),
plot.title = element_text(face = "bold", size = 18, hjust = 0.5, color = "lightgrey"),
axis.title = element_text(face = "bold", size = 14, color = "lightgrey"),
axis.text = element_text(color = "lightgrey"),
panel.grid.major = element_line(color = "lightgrey"),
panel.grid.minor = element_line(color = "lightgrey"), axis.line.x = element_line(color = "black")) +
```



```
#BOX PLOT

#finding mean of primary
mean_prim <- mean(main_df$ENSG00000001460.18[main_df$tumor_descriptor == "Primary"], na.rm = TRUE)

#finding mean of metastatic
mean_met <- mean(main_df$ENSG00000001460.18[main_df$tumor_descriptor == "Metastatic"], na.rm = TRUE)

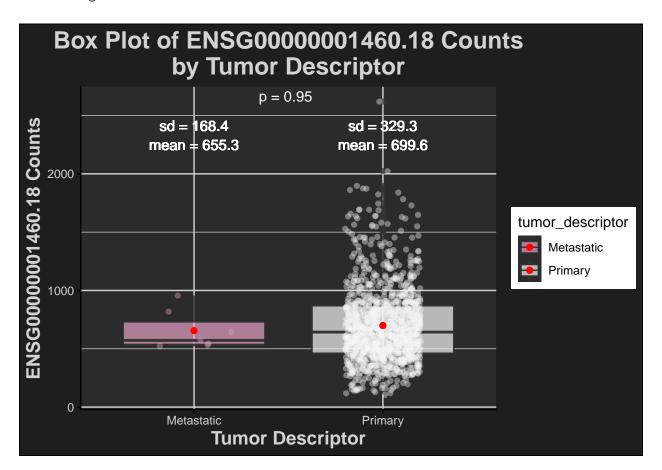
#sd of primary
sd_prim <- sd(main_df$ENSG00000001460.18[main_df$tumor_descriptor == "Primary"], na.rm = TRUE)

#sd of metastatic</pre>
```

```
sd_met <- sd(main_df$ENSG00000001460.18[main_df$tumor_descriptor == "Metastatic"], na.rm = TRUE)
#box plot of the same violin plot above
   p1 <- ggplot(main_df, aes(x = tumor_descriptor, y = ENSG00000001460.18, fill = tumor_descriptor)) +
       geom_boxplot(alpha = 0.7) + geom_jitter(aes(color = tumor_descriptor), width = 0.2, alpha = 0.4, si
#adding mean point here
       stat_summary(fun = mean, geom = "point", shape = 20, size = 3, color = "red") +
      labs(title = paste("Box Plot of ENSG00000001460.18 Counts\nby Tumor Descriptor"),
                x = "Tumor Descriptor",
                y = "ENSG00000001460.18 Counts") + theme_classic() + theme(
    plot.background = element_rect(fill = "#1e1e1e", color = "black"),
   panel.background = element_rect(fill = "#2b2b2b", color = NA),
   plot.title = element_text(face = "bold", size = 18, hjust = 0.5, color = "lightgrey"),
   axis.title = element_text(face = "bold", size = 14, color = "lightgrey"),
   axis.text = element_text(color = "lightgrey"),
   panel.grid.major = element_line(color = "lightgrey"),
   panel.grid.minor = element_line(color = "lightgrey"), axis.line.x = element_line(color = "black")) +
#adding the p value to the plot
p2 <- p1 + stat_compare_means(method = "wilcox", label = "p.format",color = "white", label.x = 1.4, siz
#looked up and figured out geom_text from https://stackoverflow.com/questions/53799878/how-to-format-gg
p3 <- p2 +
     #metastatic labels
   geom_text(aes(x = 1, y = Inf),
                     label = paste0("mean = ", round(mean_met, 1)),
                     vjust = 6, hjust = 0.5, size = 4, color = "white", inherit.aes = FALSE) +
   geom_text(aes(x = 1, y = Inf),
                     label = paste0("sd = ", round(sd_met, 1)),
                     vjust = 4.2, hjust = 0.5, size = 4, color = "white", inherit.aes = FALSE) +
   #primary labels
   geom_text(aes(x = 2, y = Inf),
                     label = paste0("mean = ", round(mean_prim, 1)),
                     vjust = 6, hjust = 0.5, size = 4, color = "white", inherit.aes = FALSE) +
   geom_text(aes(x = 2, y = Inf),
                     label = paste0("sd = ", round(sd_prim, 1)),
                     vjust = 4.2, hjust = 0.5, size = 4, color = "white", inherit.aes = FALSE)
#final boxplot
рЗ
## Warning in geom_text(aes(x = 1, y = Inf), label = paste0("mean = ", round(mean_met, : All aesthetics
## i Please consider using 'annotate()' or provide this layer with data containing
        a single row.
\#\# Warning in geom_text(aes(x = 1, y = Inf), label = pasteO("sd = ", round(sd_met, : All aesthetics have below the state of the stat
## i Please consider using 'annotate()' or provide this layer with data containing
      a single row.
## Warning in geom_text(aes(x = 2, y = Inf), label = paste0("mean = ", round(mean_prim, : All aesthetic
```

```
## i Please consider using 'annotate()' or provide this layer with data containing
## a single row.
```

Warning in geom_text(aes(x = 2, y = Inf), label = pasteO("sd = ", round(sd_prim, : All aesthetics ha
i Please consider using 'annotate()' or provide this layer with data containing
a single row.



```
#heatmap libraries are at the top

#I found this package and the functions within it to help me resize my heat map via https://stackoverfl

#selecting 10 genes
genes_to_plot <- colnames(main_df)[11:20]
head(genes_to_plot)

## [1] "ENSG00000001167.14" "ENSG00000001460.18" "ENSG000000001461.17"

## [4] "ENSG00000001497.18" "ENSG00000001561.7" "ENSG000000001617.12"

#subsetting those genes
counts_subset <- main_df[,genes_to_plot]

#making sure to transpose them back into rows for the heat map
counts_subset <- t(counts_subset)</pre>
```

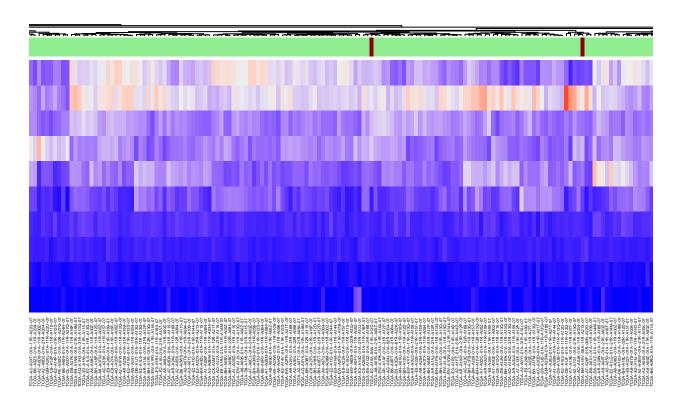
```
#adding back samples
colnames(counts_subset) <- main_df$barcode</pre>
#heat map annotaitons
column_ha <- HeatmapAnnotation(</pre>
 tumor_descriptor = main_df$tumor_descriptor,
  col = list(
   tumor_descriptor = c(Primary = "lightgreen", Metastatic = "darkred", `Not Applicable` = "darkgrey")
#create heat map
hm <-Heatmap(</pre>
  counts_subset,
 name = "Counts",
 top_annotation = column_ha,
  show_row_names = TRUE,
  show_column_names = TRUE,
  column_names_gp = grid::gpar(fontsize= 3),
  column_names_centered = TRUE,
  cluster columns = TRUE,
  cluster rows = TRUE,
 width = unit(120, "cm"), #changing the width since its so many samples
 column_title = "Samples",
 row_title = "Genes",
 heatmap_legend_param = list(title = "Count")
## The automatically generated colors map from the 1^st and 99^th of the
## values in the matrix. There are outliers in the matrix whose patterns
## might be hidden by this color mapping. You can manually set the color
## to 'col' argument.
##
## Use 'suppressMessages()' to turn off this message.
#creating a heat map without samples for smaller visual
hm_no_samples <- Heatmap(</pre>
 counts subset,
 name = "Counts",
 top_annotation = column_ha,
  show_row_names = TRUE,
  show_column_names = FALSE,
  cluster_columns = TRUE,
  cluster_rows = TRUE,
  width = unit(6, "cm"),
  column_title = "Samples",
 row_title = "Genes",
 heatmap_legend_param = list(title = "Count"))
```

The automatically generated colors map from the 1°st and 99°th of the ## values in the matrix. There are outliers in the matrix whose patterns ## might be hidden by this color mapping. You can manually set the color ## to 'col' argument.

##
Use 'suppressMessages()' to turn off this message.

#PLEASE READ: MY HEATMAP IS SO LARGE YOU HAVE TO DOWNLOAD AS PNG TO SEE IT. PLEASE SEE ATTACHED IN ASSI

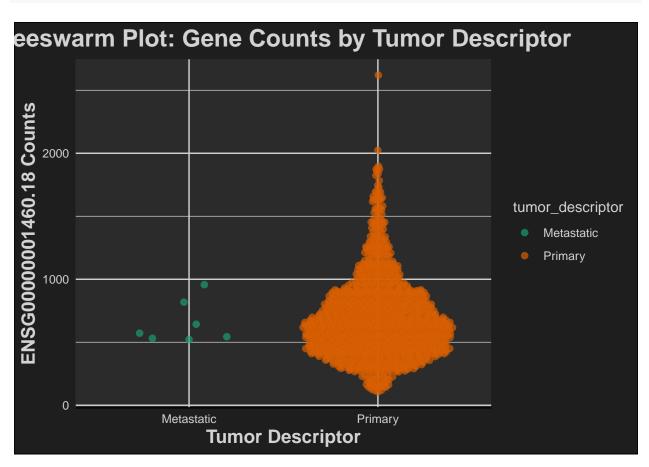
Samples



 ${\tt hm_no_samples}$

```
Samples
                                      tumor_descriptor
                                      ENSG00000001617.12
                                      ENSG00000001629.10
                                      ENSG0000001167.14
                                      ENSG0000001497.18
                                                              Count
                                                                      tumor_descripto
                                                                15000
                                                                        Metastatic
                                      ENSG00000001461.17
                                                                       Primary
                                                                10000
                                      ENSG00000001561.7
                                                                5000
                                      ENSG00000001460.18
                                      ENSG00000001631.16
                                      ENSG0000001630.17
                                      ENSG00000001626.16
png("myheatmap-final.png", width = 17000, height = 2100, res = 300)
hm
dev.off()
## pdf
##
    2
png("myheatmap-final_no_colnames.png")
hm_no_samples
dev.off()
## pdf
##
#BEESWARM PLOT
#loading package (ggbeeswarm) at top
#making a beeswarm plot of the same gene and tumor description to see density
ggplot(main_df, aes(x = tumor_descriptor, y = ENSG00000001460.18, color = tumor_descriptor)) +
 geom_quasirandom(dodge.width = 0.75, size = 2, alpha = 0.7) + theme_minimal() +
  plot.background = element_rect(fill = "#1e1e1e", color = "black"),
 panel.background = element_rect(fill = "#2b2b2b", color = NA),
```

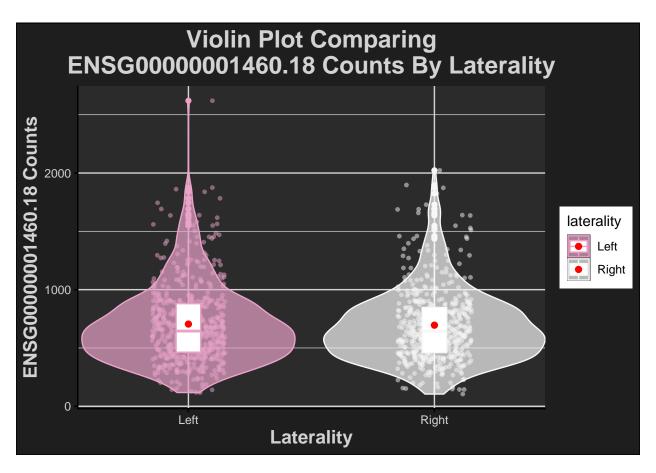
```
plot.title = element_text(face = "bold", size = 18, hjust = 0.5, color = "lightgrey"),legend.text = e
axis.title = element_text(face = "bold", size = 14, color = "lightgrey"),
axis.text = element_text(color = "lightgrey"),
panel.grid.major = element_line(color = "lightgrey"),
panel.grid.minor = element_line(color = "lightgrey"), axis.line.x = element_line(color = "black")) +
labs(x = "Tumor Descriptor", y = "ENSG000000001460.18 Counts", title = "Beeswarm Plot: Gene Counts by scale_color_brewer(palette = "Dark2")
```



```
#VIOLIN PLOT DIFFERENT COVARIATE

#getting rid of NAs
main_df <- main_df[(is.na(main_df$laterality) == FALSE),]

#putting together the violin plot of laterality for my gene
ggplot(main_df, aes(x = laterality, y = ENSG00000001460.18 , fill = laterality, color = laterality))+
    geom_violin(alpha =0.7, position = position_dodge(width = 2)) + geom_jitter(width = 0.15, size = 1, a
        stat_summary(fun = mean, geom = "point", shape = 20, size = 3, color = "red") +
    labs(x = "Laterality", y = "ENSG0000001460.18 Counts", title = "Violin Plot Comparing\nENSG000000014
    plot.background = element_rect(fill = "#1e1e1e", color = "black"),
    panel.background = element_rect(fill = "#2b2b2b", color = NA),
    plot.title = element_text(face = "bold", size = 18, hjust = 0.5, color = "lightgrey"),
    axis.tttle = element_text(face = "bold", size = 14, color = "lightgrey"),
    axis.text = element_text(color = "lightgrey"),
    panel.grid.major = element_line(color = "lightgrey"),
    panel.grid.minor = element_line(color = "lightgrey"), axis.line.x = element_line(color = "black")) +</pre>
```



```
#RAINDROP PLOT
#loading library library(ggdist) at the top
#qetting rid of NAs
main_df <- main_df[(is.na(main_df$vital_status) == FALSE),]</pre>
main_df <- main_df[(is.na(main_df$gender) == FALSE),]</pre>
#creating a half eye/rain cloud plot
ggplot(main_df, aes(x = gender, y = ENSG00000001460.18, fill = vital_status)) +
  stat halfeye(aes(color = vital status), position = position dodge(width = 0.75), alpha = 0.6) +
  geom_boxplot(width = 0.2, position = position_dodge(width = 0.75), outlier.shape = NA, alpha = 0.7) +
  geom_jitter(aes(color = vital_status), position = position_dodge(width = 0.75), size = 1.5, alpha = 0
  theme_minimal() + theme(
  plot.background = element_rect(fill = "#1e1e1e", color = "black"),
  panel.background = element_rect(fill = "#2b2b2b", color = NA),
  plot.title = element_text(face = "bold", size = 18, hjust = 0.5, color = "lightgrey"), legend.title =
  axis.title = element_text(face = "bold", size = 14, color = "lightgrey"),
  axis.text = element_text(color = "lightgrey"),
  panel.grid.major = element_line(color = "lightgrey"),
  panel.grid.minor = element_line(color = "lightgrey"), axis.line.x = element_line(color = "black")) +
  labs(title = "Raincloud Plot: ENSG00000001460.18 Counts\nby Gender & Vital Status") +
  scale_fill_brewer(palette = "Accent") +
  scale_color_brewer(palette = "Accent")
```

