MDS_sexCheck.R

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```
library(limma)
library(edgeR)
```

```
## Warning: package 'edgeR' was built under R version 3.6.1
```

```
library(RColorBrewer)
library(ggplot2)
```

```
## Warning: package 'ggplot2' was built under R version 3.6.2
```

```
library(tidyverse)
```

```
## -- Attaching packages ------------------------------------------------- tidyverse 1.3.0 --
```

```
## v tibble  3.0.3     v dplyr   1.0.1
## v tidyr   1.1.1     v stringr 1.4.0
## v readr   1.3.1     v forcats 0.5.0
## v purrr   0.3.4
```

```
## Warning: package 'tibble' was built under R version 3.6.2
```

```
## Warning: package 'tidyr' was built under R version 3.6.2
```

```
## Warning: package 'purrr' was built under R version 3.6.2
```

```
## Warning: package 'dplyr' was built under R version 3.6.2
```

```
## -- Conflicts ---------------------------------------------------- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
library(matrixStats)
```

```
##
## Attaching package: 'matrixStats'
```

```
## The following object is masked from 'package:dplyr':
##
##     count
```

```
library(reshape)
```

```
##
## Attaching package: 'reshape'
```

```
## The following object is masked from 'package:dplyr':
##
##     rename
```

```
## The following objects are masked from 'package:tidyr':
```

```
##
##     expand, smiths
```

```
library(ggpubr)
```

```
## Warning: package 'ggpubr' was built under R version 3.6.2
```

```
library("gridExtra")
```

```
##
## Attaching package: 'gridExtra'
```

```
## The following object is masked from 'package:dplyr':
##
##     combine
```

```
# set working directory
```

```
#setwd("~/Dropbox (ASU)/Placenta/BATCH2_PLACENTA_DECIDUA_ANALYSIS/HISAT_FeatureCounts/batch1_and_batch2/
```

```
# Read in count, genes, and phenotype data
```

```
counts_g <- read.delim("counts_pheno/placenta_batch1and2_geneCounts.tsv", header=TRUE, sep="\t")
colnames(counts_g) <- str_replace_all(colnames(counts_g), pattern="\\.","-") # replace . with - in samp
genes <- read.csv("counts_pheno/genesID.csv", header=TRUE, sep = ",")

counts_t <- read.delim("counts_pheno/placenta_batch1and2_transcriptCounts.tsv", header=TRUE, sep="\t")
colnames(counts_t) <- str_replace_all(colnames(counts_t), pattern="\\.","-") # replace . with - in samp
transcripts <- read.csv("counts_pheno/transcriptsID.csv", header=TRUE, sep = ",")

#pheno <- read.csv("counts_pheno/placenta_pheno.txt", header=TRUE, sep="\t")
pheno <- read.csv("counts_pheno/200508_placentas_pheno.csv", header=TRUE, sep=",")

placenta_batch1_sampleIDs <- c("OBG0044-1", "OBG0044-2", "OBG0053-1", "OBG0053-2", "OBG0068-1",
                               "OBG0068-2", "OBG0111-1", "OBG0111-2", "OBG0112-1", "OBG0112-2",
                               "OBG0115-1", "OBG0115-2", "OBG0116-1", "OBG0116-2", "OBG0117-1",
                               "OBG0117-2", "OBG0118-1", "OBG0118-2", "OBG0120-1", "OBG0120-2",
                               "OBG0122-1", "OBG0122-2", "OBG0123-1", "OBG0123-2", "OBG0126-1",
                               "OBG0126-2", "OBG0130-1", "OBG0130-2", "OBG0132-1", "OBG0132-2",
                               "OBG0133-1", "OBG0133-2", "OBG0156-1", "OBG0156-2", "OBG0158-1",
                               "OBG0158-2", "OBG0166-1", "OBG0166-2", "OBG0170-1", "OBG0170-2",
                               "OBG0174-1", "OBG0174-2", "OBG0175-1", "OBG0175-2", "OBG0178-1",
                               "OBG0178-2", "YPOPS0006-1", "YPOPS0006-2")

# batch 2 samples
placenta_batch2_sampleIDs <- c("OBG0014-1", "OBG0014-2", "OBG0015-1", "OBG0015-2", "OBG0019-1",
                               "OBG0019-2", "OBG0021-1", "OBG0021-2", "OBG0022-1", "OBG0022-2",
                               "OBG0024-1", "OBG0024-2", "OBG0026-1", "OBG0026-2", "OBG0027-1",
                               "OBG0027-2", "OBG0028-1", "OBG0028-2", "OBG0029-1", "OBG0029-2",
                               "OBG0030-1", "OBG0030-2", "OBG0031-1", "OBG0031-2", "OBG0032-1",
                               "OBG0032-2", "OBG0039-1", "OBG0039-2", "OBG0047-1", "OBG0047-2",
                               "OBG0050-1", "OBG0050-2", "OBG0051-1", "OBG0051-2", "OBG0053B2-1",
                               "OBG0053B2-2", "OBG0065-1", "OBG0065-2", "OBG0066-1", "OBG0066-2",
                               "OBG0085-1", "OBG0085-2", "OBG0090-1","OBG0090-2", "OBG0107-1",
                               "OBG0107-2", "OBG0121-1", "OBG0121-2", "OBG0138-1", "OBG0138-2",
                               "OBG0149-1", "OBG0149-2", "OBG0180-1", "OBG0180-2", "OBG0188-1",
                               "OBG0188-2", "OBG0191-1", "OBG0191-2", "OBG0201-1", "OBG0201-2",
```

```
                                "OBG0205-1", "OBG0205-2", "OBG0289-1", "OBG0289-2", "OBG0338-1",
                                "OBG0338-2", "OBG0342-1", "OBG0342-2", "YPOPS0007M-1", "YPOPS0007M-2",
                                "YPOPS0123M-1", "YPOPS0123M-2")

# samples to remove due to failed QC and/or outlier in MDS plot
placenta_batch1_removals <- c("OBG0174-1", "OBG0174-2", "OBG0175-1", "OBG0175-2")
placenta_batch2_removals <- c("OBG0015-1", "OBG0015-2", "OBG0065-1", "OBG0065-2",
                              "OBG0188-1", "OBG0188-2", "OBG0014-1", "OBG0014-2",
                              "OBG0026-1", "OBG0026-2", "YPOPS0007M-1","YPOPS0007M-2",
                              "OBG0019-1", "OBG0019-2", "OBG0021-1", "OBG0021-2")

placenta_removals <- c(placenta_batch2_sampleIDs)
all_removals <- c(placenta_removals)

samplesToRemove <- c(all_removals) # update depending on comparison being made
SAMPLE_LENGTH <- as.numeric(length(samplesToRemove)) # to call later
half_sample_length <- SAMPLE_LENGTH/2 # half the sample length

removals_g <- (names(counts_g) %in% samplesToRemove[1:SAMPLE_LENGTH]) # for matching names create a val
counts_ExRemovals_g <-counts_g[!removals_g] # create a new counts file that excludes (Ex) the removals

removals_t <- (names(counts_t) %in% samplesToRemove[1:SAMPLE_LENGTH]) # for matching names create a val
counts_ExRemovals_t <-counts_t[!removals_t] # create a new counts file that excludes (Ex) the removals
pheno_ExRemovals <- pheno[! pheno$sample %in% samplesToRemove[1:SAMPLE_LENGTH],] # update 1:16 dependin
```

```
# create a DGElist
```

```
dge_g <- DGEList(counts=counts_ExRemovals_g, genes=genes)
dge_t <- DGEList(counts=counts_ExRemovals_t, genes=transcripts)
dim(dge_g)
```

```
## [1] 57133    48
```

```
dim(dge_t)
```

```
## [1] 206694     48
```

```
# organize sample information
```

```
samplenames <- (pheno_ExRemovals$sample) # sample names are not unique because a sample may belong to m
as.data.frame(samplenames)
```

```
##    samplenames
## 1    OBG0044-1
## 2    OBG0044-2
## 3    OBG0053-1
## 4    OBG0053-2
## 5    OBG0068-1
## 6    OBG0068-2
## 7    OBG0111-1
## 8    OBG0111-2
## 9    OBG0112-1
## 10   OBG0112-2
## 11   OBG0115-1
## 12   OBG0115-2
## 13   OBG0116-1
```

```
## 14   OBG0116-2
## 15   OBG0117-1
## 16   OBG0117-2
## 17   OBG0118-1
## 18   OBG0118-2
## 19   OBG0120-1
## 20   OBG0120-2
## 21   OBG0122-1
## 22   OBG0122-2
## 23   OBG0123-1
## 24   OBG0123-2
## 25   OBG0126-1
## 26   OBG0126-2
## 27   OBG0130-1
## 28   OBG0130-2
## 29   OBG0132-1
## 30   OBG0132-2
## 31   OBG0133-1
## 32   OBG0133-2
## 33   OBG0156-1
## 34   OBG0156-2
## 35   OBG0158-1
## 36   OBG0158-2
## 37   OBG0166-1
## 38   OBG0166-2
## 39   OBG0170-1
## 40   OBG0170-2
## 41   OBG0174-1
## 42   OBG0174-2
## 43   OBG0175-1
## 44   OBG0175-2
## 45   OBG0178-1
## 46   OBG0178-2
## 47 YPOPS0006-1
## 48 YPOPS0006-2
```

```
colnames(dge_g) <- samplenames
colnames(dge_t) <- samplenames

# create groups for the samples
sex <- factor(pheno_ExRemovals$sex, levels=c("female", "male"))
batch<- factor(pheno_ExRemovals$batch, levels=c("1", "2"))

dge_g$samples$sex <- sex
dge_g$samples$batch <- batch

dge_t$samples$sex <- sex
dge_t$samples$batch <- batch
```

```
# data pre-processing
```

```
#dge_g <-sumTechReps(dge_g, dge_g$samples$rep) # comment out to not sum replicates
#dge_t <-sumTechReps(dge_t, dge_t$samples$rep) # comment out to not sum replicates
```

```
cpm_gene <- cpm(dge_g) #log2-transformed counts per gene per million mapped reads (cpm).
lcpm_gene <- cpm(cpm_gene, log=TRUE)

cpm_tran <- cpm(dge_t) #log2-transformed counts per gene per million mapped reads (cpm).
lcpm_tran <- cpm(cpm_tran, log=TRUE)
```

```
sex <- dge_g$samples$sex
batch <- dge_g$samples$batch
```

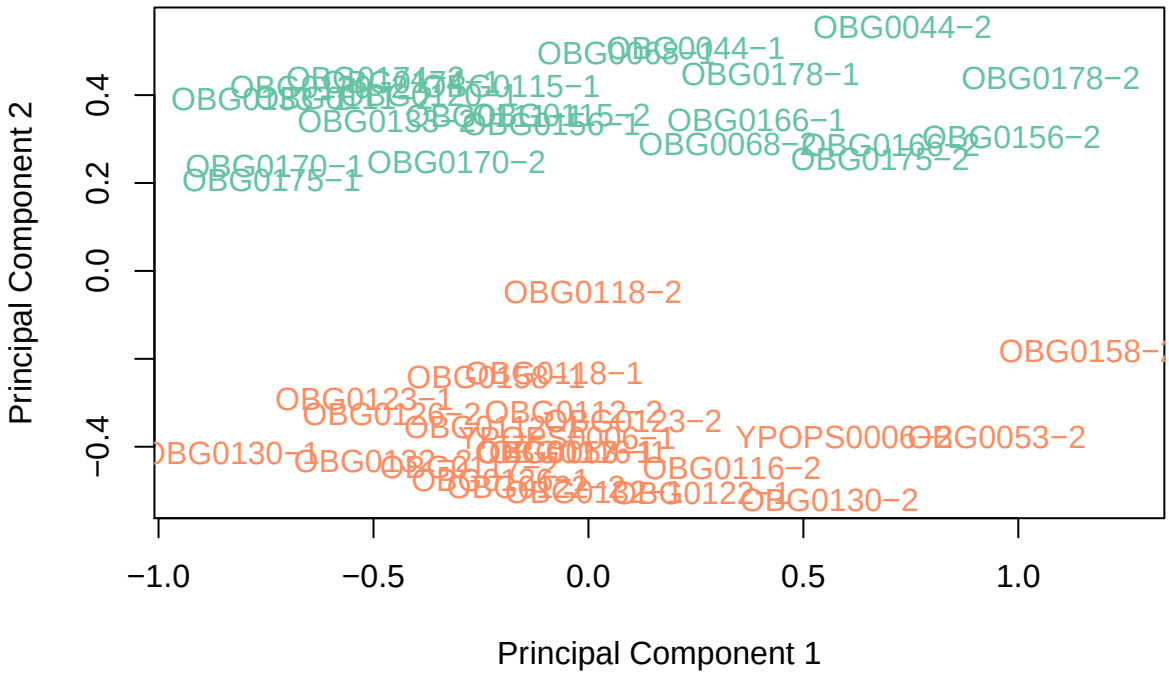
```
col.sex <- sex
levels(col.sex) <-  brewer.pal(nlevels(col.sex), "Set2")
```

```
## Warning in brewer.pal(nlevels(col.sex), "Set2"): minimal value for n is 3, returning requested palet
```

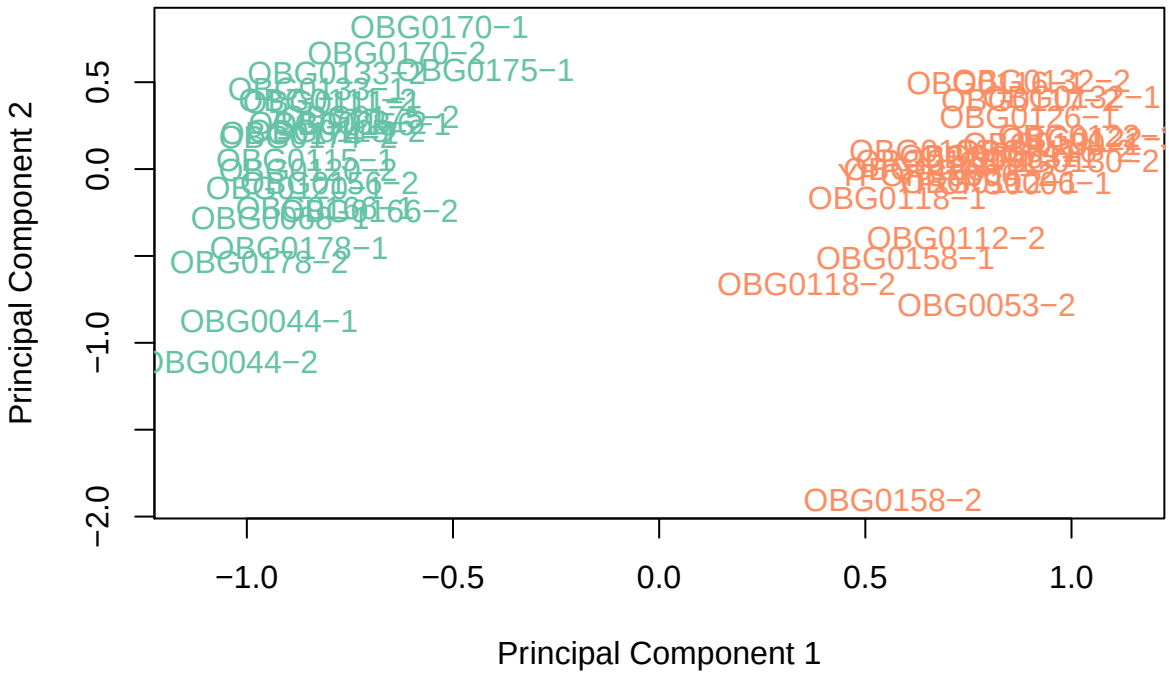
```
col.sex <- as.character(col.sex)
```

```
# plot MDS
# If gene.selection is "common", then the top genes are those with the largest standard deviations betw
```

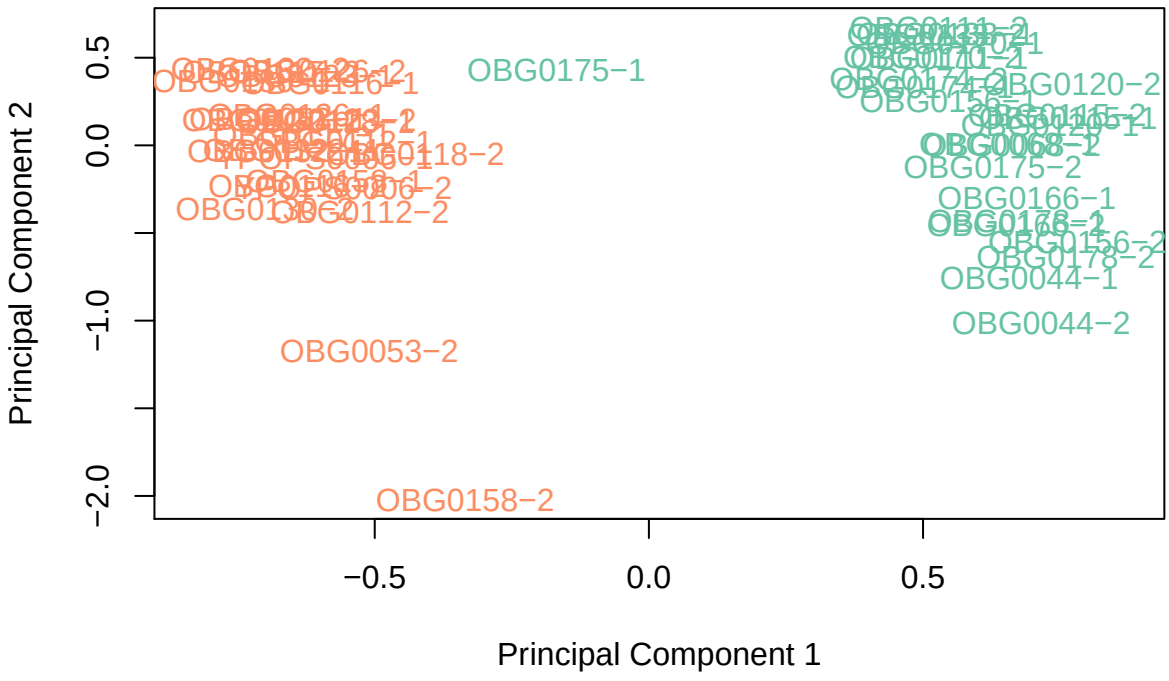
```
# all genes
plotMDS(lcpm_gene, col=col.sex,
        gene.selection = "common", dim.plot = c(1,2))
```



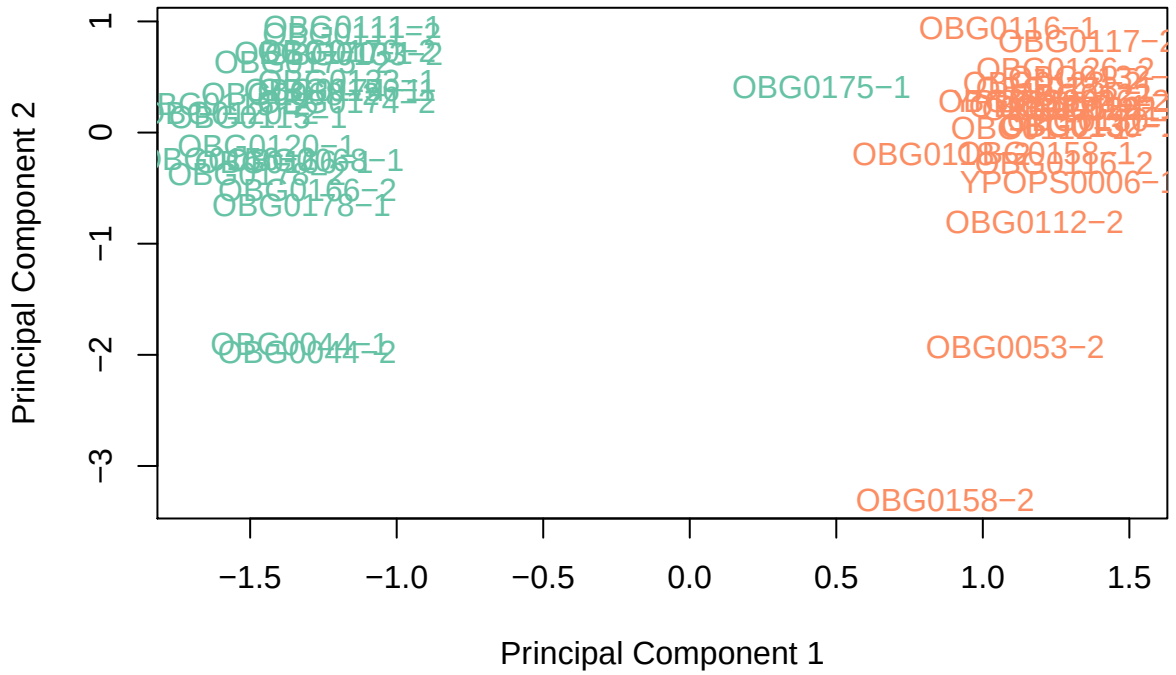

```
# top 100 genes
plotMDS(lcpm_gene, col=col.sex,
        top = 100,
        gene.selection = "common", dim.plot = c(1,2))
```




```
# all transcripts
plotMDS(lcpm_tran, col=col.sex,
        gene.selection = "common", dim.plot = c(1,2))
```




```
# top 100 transcripts
plotMDS(lcpm_tran, col=col.sex,
        top = 100,
        gene.selection = "common", dim.plot = c(1,2))
```




```
may <- cbind(genes, cpm_gene)
may$length <- NULL
may2 <- melt(may, id=c("Geneid", "chr"))
df_merged <- merge(may2, pheno_ExRemovals, by.x="variable", by.y="sample", all.x=TRUE)
df <- df_merged[ -c(5,7:21) ]
head(df)
```

```
##    variable      Geneid  chr      value    sex
## 1 OBG0044-1     DDX11L1 chr1 0.16879918 female
## 2 OBG0044-1      WASH7P chr1 5.85170497 female
## 3 OBG0044-1   MIR6859-1 chr1 0.22506558 female
## 4 OBG0044-1 MIR1302-2HG chr1 0.05626639 female
## 5 OBG0044-1   MIR1302-2 chr1 0.00000000 female
## 6 OBG0044-1     FAM138A chr1 0.00000000 female
```

```
gametology <- read.delim("gametology/gametology.txt", header = TRUE)

# make an X and Y chromosome list of the gametology genes
gametology_inter_X <- intersect(gametology$X, df$Geneid)
gametology_inter_Y <- intersect(gametology$Y, df$Geneid)
# what is shared between the two lists
gametology_inter <- unique(c(gametology_inter_X, gametology_inter_Y))

# subset the placent CPM data to only incldue the X and Y gametology genes
placenta_gametologys <- subset(df, Geneid %in% gametology_inter)
```

```
# for loop to format the placenta CPM into
# the format needed for making plots
DF_null <- data.frame()
geneDF <- data.frame()
geneComb <- NULL
groupComb <- NULL
variabledf <- NULL
```

```r
Geneiddf <- NULL
sexdf <- NULL
CPM <- NULL


for(i in gametology_inter) {
  geneDF <- subset(placenta_gametologys, Geneid == i)
  variabledf <- c(variabledf, as.character(geneDF$variable))
  Geneiddf <- c(Geneiddf, as.character(geneDF$Geneid))
  sexdf <- c(sexdf, as.character(geneDF$sex))
  CPM <- c(CPM, as.numeric(geneDF$value))
  Y <- sub("X$", "Y", as.character(geneDF$Geneid))
  groupComb <- paste0(as.character(geneDF$Geneid),":",Y)
  geneComb <- c(geneComb, as.character(groupComb))
  DF_null <- cbind(variabledf, Geneiddf, sexdf, CPM, geneComb)
}

# save as a data frame
# rename the gene groups
placenta_gametologys_df <- as.data.frame(DF_null)
names(placenta_gametologys_df)[names(placenta_gametologys_df) == "sexdf"] <- "sex"
names(placenta_gametologys_df)[names(placenta_gametologys_df) == "variabledf"] <- "sample"
names(placenta_gametologys_df)[names(placenta_gametologys_df) == "Geneiddf"] <- "gene"
placenta_gametologys_df$geneComb <- as.character(placenta_gametologys_df$geneComb)
placenta_gametologys_df[placenta_gametologys_df=="DDX3Y:DDX3Y"] <- "DDX3X:DDX3Y"
placenta_gametologys_df[placenta_gametologys_df=="PCDH11Y:PCDH11Y"] <- "PCDH11X:PCDH11Y"
placenta_gametologys_df[placenta_gametologys_df=="USP9Y:USP9Y"] <- "USP9X:USP9Y"
placenta_gametologys_df[placenta_gametologys_df=="ZFY:ZFY"] <- "ZFX:ZFY"
placenta_gametologys_df[placenta_gametologys_df=="KDM6A:UTY"] <- "UTX:UTY"
placenta_gametologys_df[placenta_gametologys_df=="KDM6A:KDM6A"] <- "UTX:UTY"
placenta_gametologys_df[placenta_gametologys_df=="UTY:UTY"] <- "UTX:UTY"
```

```r
# subset the placenta_gametologys_df by male and female
male <- subset(placenta_gametologys_df, sex == "male")
female <- subset(placenta_gametologys_df, sex == "female")

# only X chromosome linked genes
female_X <- subset(female, gene %in% gametology_inter_X)
male_X <- subset(male, gene %in% gametology_inter_X)

# only Y chromosome linked genes
male_Y <- subset(male, gene %in% gametology_inter_Y)
female_Y <- subset(female, gene %in% gametology_inter_Y)

# Merge the male_X with male_Y
# this will be used to then sum the X and Y expression for each sample and each gene
maleXandY <- merge(x = male_X, y = male_Y, by = c("sample", "geneComb"), all = TRUE) #, by.y = names(ge

# the X and Y gametology genes don't match since there is SRY and XIST
# this creates NAs
# replace NAs with zero
maleXandY[is.na(maleXandY)] <- 0
```

```
## Warning in `[<-.factor`(`*tmp*`, thisvar, value = 0): invalid factor level, NA
```

```
## generated

## Warning in `[<-.factor`(`*tmp*`, thisvar, value = 0): invalid factor level, NA
## generated

## Warning in `[<-.factor`(`*tmp*`, thisvar, value = 0): invalid factor level, NA
## generated

## Warning in `[<-.factor`(`*tmp*`, thisvar, value = 0): invalid factor level, NA
## generated
```

```
# warning messages are okay

# sum the X and Y CPM values
maleXandY$CPM <- as.numeric(as.character(maleXandY$CPM.x)) + as.numeric(as.character(maleXandY$CPM.y))
#maleXandY$CPM <- as.numeric(as.character(maleXandY$CPM.x)) + as.numeric(as.character(maleXandY$CPM.y))

# Drop the .y columns and the CPM.x
drops <- c("CPM.x", "CPM.y","gene.y", "sex.y", "CPM.y")
maleDrops <- maleXandY[ , !(names(maleXandY) %in% drops)]

maledf <- as.data.frame(maleDrops)
# rename so of the .x columns
male_rename <- rename(maledf, c("gene.x"="gene", "sex.x"="sex"))
# fill in missing male information
male_rename$sex[is.na(male_rename$sex)] <- "male"
male_XY <- male_rename[complete.cases(male_rename[ , 3:4]),]

# add back in Y chromsome names
# Y chromosome gene names
#male_rename_sry <- subset(male_rename, geneComb == "SRY:SRY")
#male_rename_sry$gene[is.na(male_rename_sry$gene)] <- "SRY"

# rbind male and female X chromosome expression information
male_female_Xchr <- rbind(male_XY, female_X)
# CPM column should be numeric
male_female_Xchr$CPM <- as.numeric(as.character(male_female_Xchr$CPM))
```

```
male_female_Xchr_PLOT <- "./FIGURES/sexCheck_geneExpression.pdf"
pdf(male_female_Xchr_PLOT)

# Function to plot histograms on top of each other
violoin_Func <- function(a) {
  geneDF <- subset(male_female_Xchr, gene == a)
  means <- aggregate(CPM ~sex, geneDF, mean)
  p <- ggplot(geneDF, aes(x = sex, y = CPM, color = sex)) +
    geom_violin() + scale_color_manual(values = c("black", "#66C2A5",  "#FC8D62")) +
    #   facet_wrap(~geneComb) + theme(strip.text.x = element_text(size = 12)) +
    geom_boxplot(width = 0.1, outlier.shape = NA) +
    geom_jitter(aes(shape = factor(sex)),
                size = 3,
                position = position_jitter(0.1)) +
    theme(legend.position = "none") + ggtitle(paste0(a, " CPM")) +
    theme(axis.title.x=element_text(size=15),
          axis.text.x=element_text(size=12)) +
```

```
    theme(axis.title.y=element_text(size=15),
          axis.text.y=element_text(size=12)) +
    theme(axis.title=element_text(size=15)) +
    theme(legend.text=element_text(size=12)) +
    theme(legend.title=element_text(size=15)) +
    geom_text(
      data = means,
      aes(
        label = round(CPM, digits = 2),
        y = CPM,
        color = "black"
      ),
      position = position_dodge(width = 0.9),
      size = 5
    ) +
    theme(axis.text = element_text(size = 5, colour="black")) +
    stat_compare_means(method = "t.test",
                       label.x = 1.2,
                       label.y.npc = 1) +
    labs(y = "CPM")
}

# Map: iterates through items in Meta (which is a list of dataframes)
# and iterates through the names of the items in Meta simultaneously
violinPlots <- Map(violoin_Func, a = gametology_inter_X)
violinPlots
```

```
## $DDX3X
```



```
##
## $PCDH11X
```



```
##
## $USP9X
```



```
##
## $ZFX
```



```
##
## $KDM6A
```



```
##
## $XIST
```



```
##
## $KDM5C
```



```
##
## $PRKX
```



```
##
## $RPS4X
```



```
##
## $EIF1AX
```



```
##
## $NLGN4X
```

```
##
## $TGIF2LX
```

```
## Warning: Computation failed in `stat_compare_means()`:
## arguments imply differing number of rows: 0, 1
```

```
##
## $SOX3
```

```
## Warning: Computation failed in `stat_compare_means()`:
## arguments imply differing number of rows: 0, 1
```

```
##
## $AMELX
```

```
##
## $TBL1X
```

```
##
## $TMSB4X
```

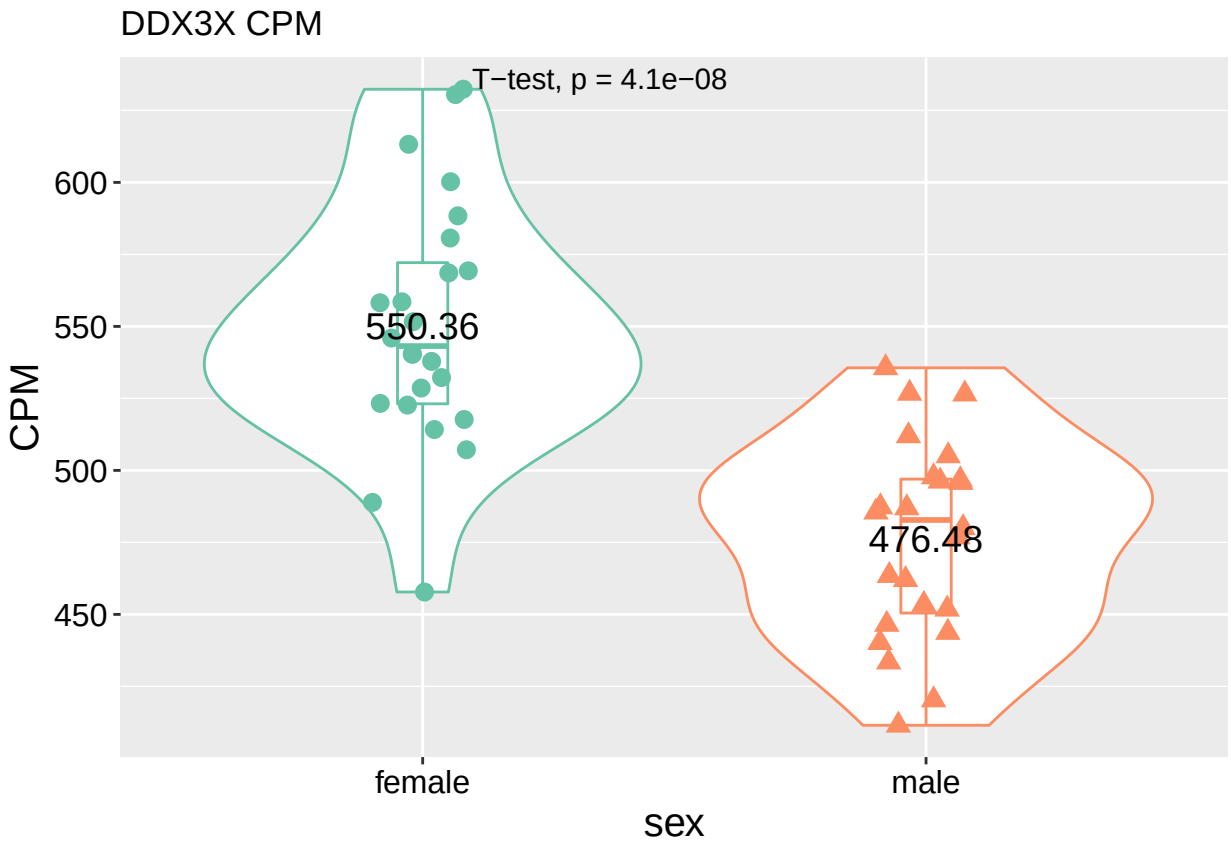
```
##
## $VCX
```

```
##
## $RBMX
```

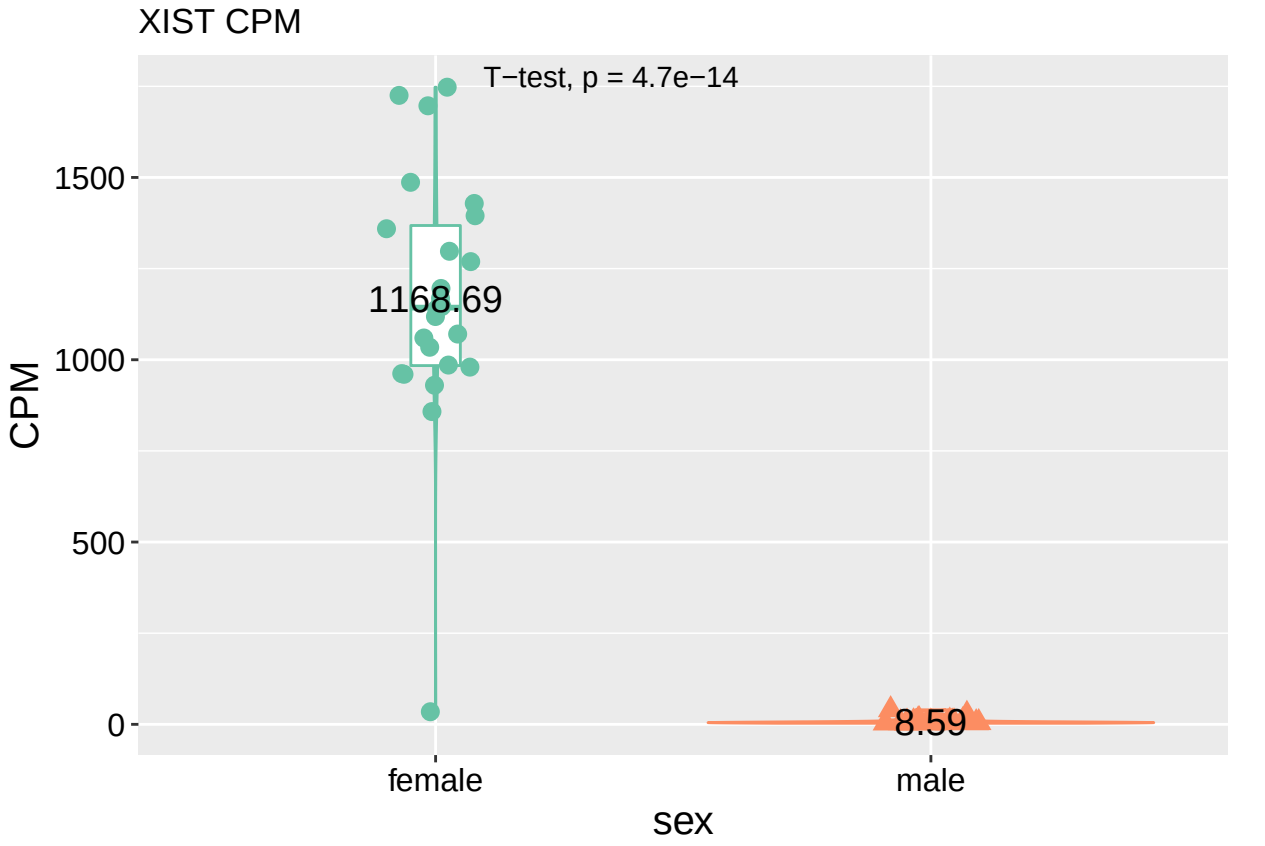
```
dev.off()
```

```
## pdf
##   2
```

```
violinPlots$DDX3X
```




```
violinPlots$XIST
```

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violinPlots$PCDH11X
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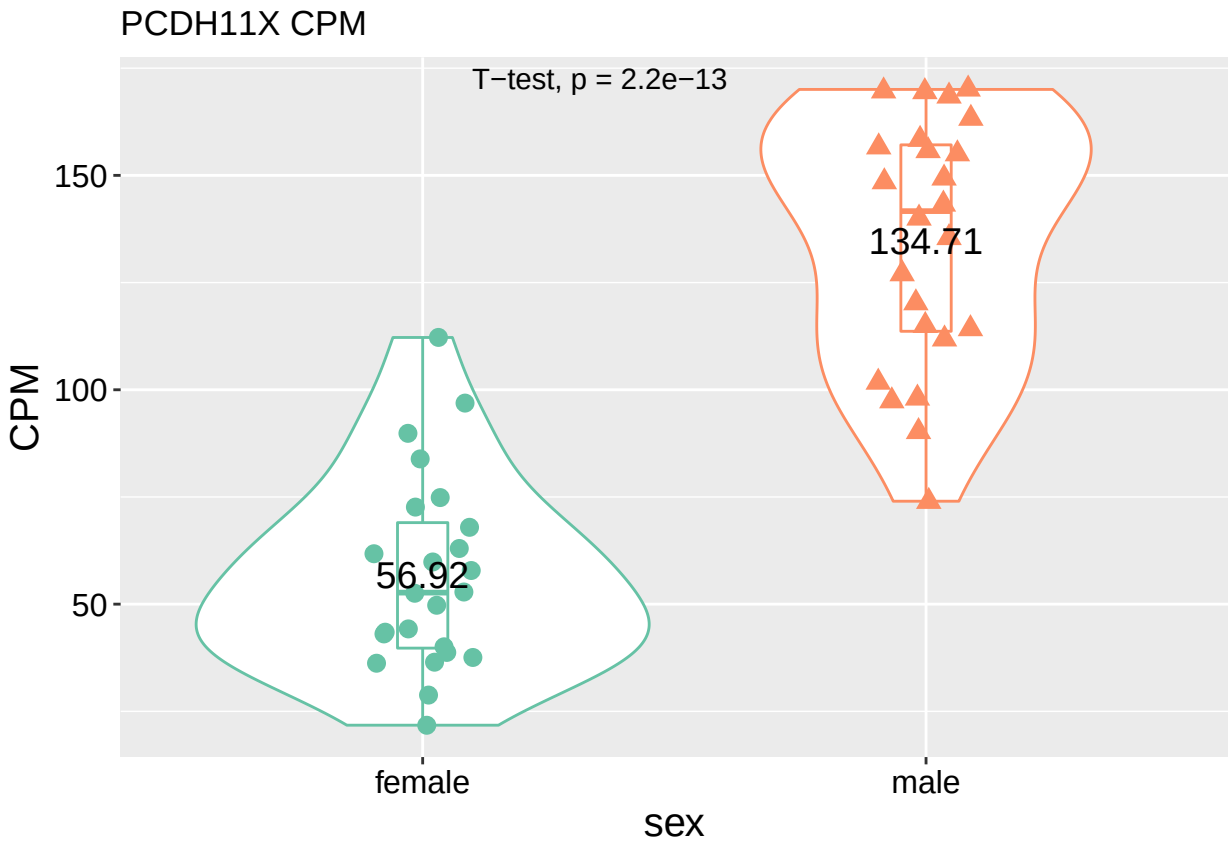


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violinPlots$ZFX
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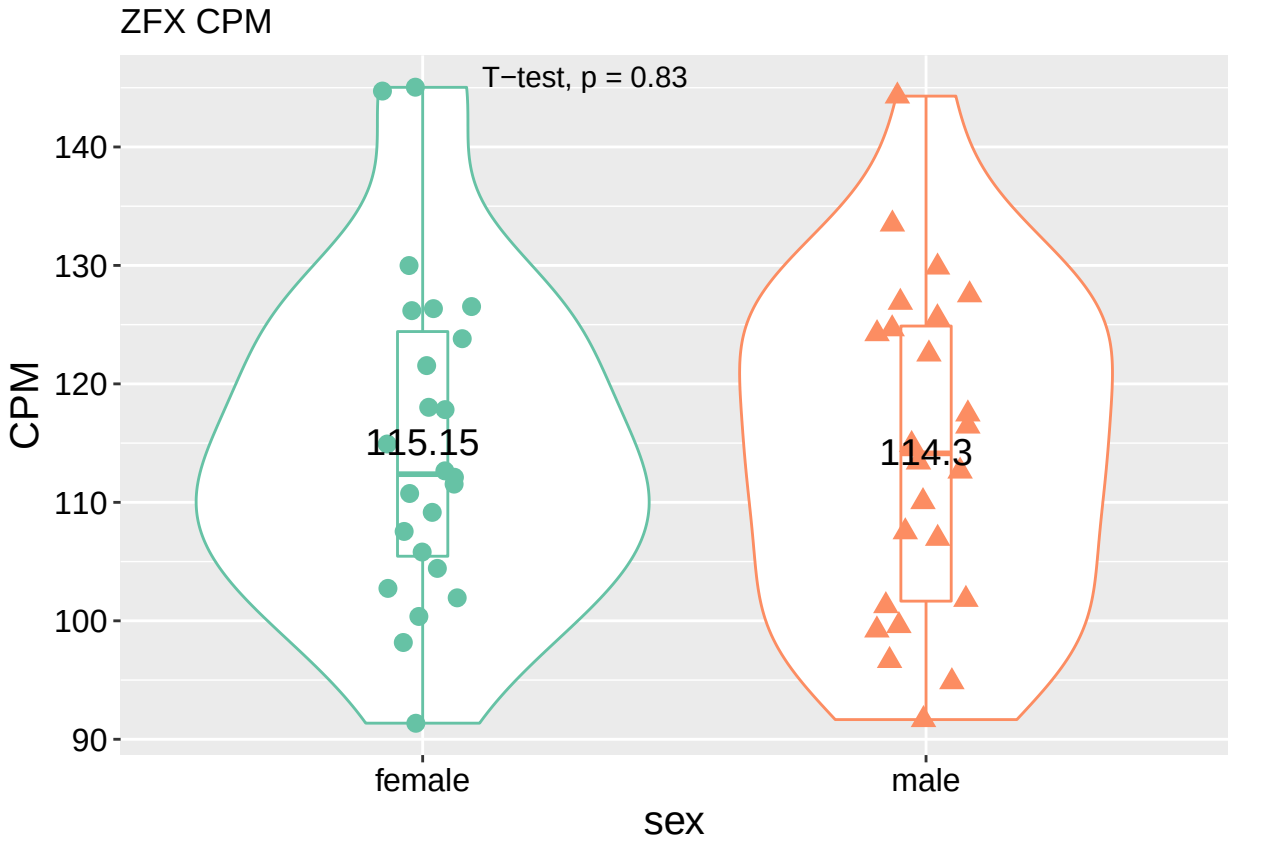


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violinPlots$USP9X
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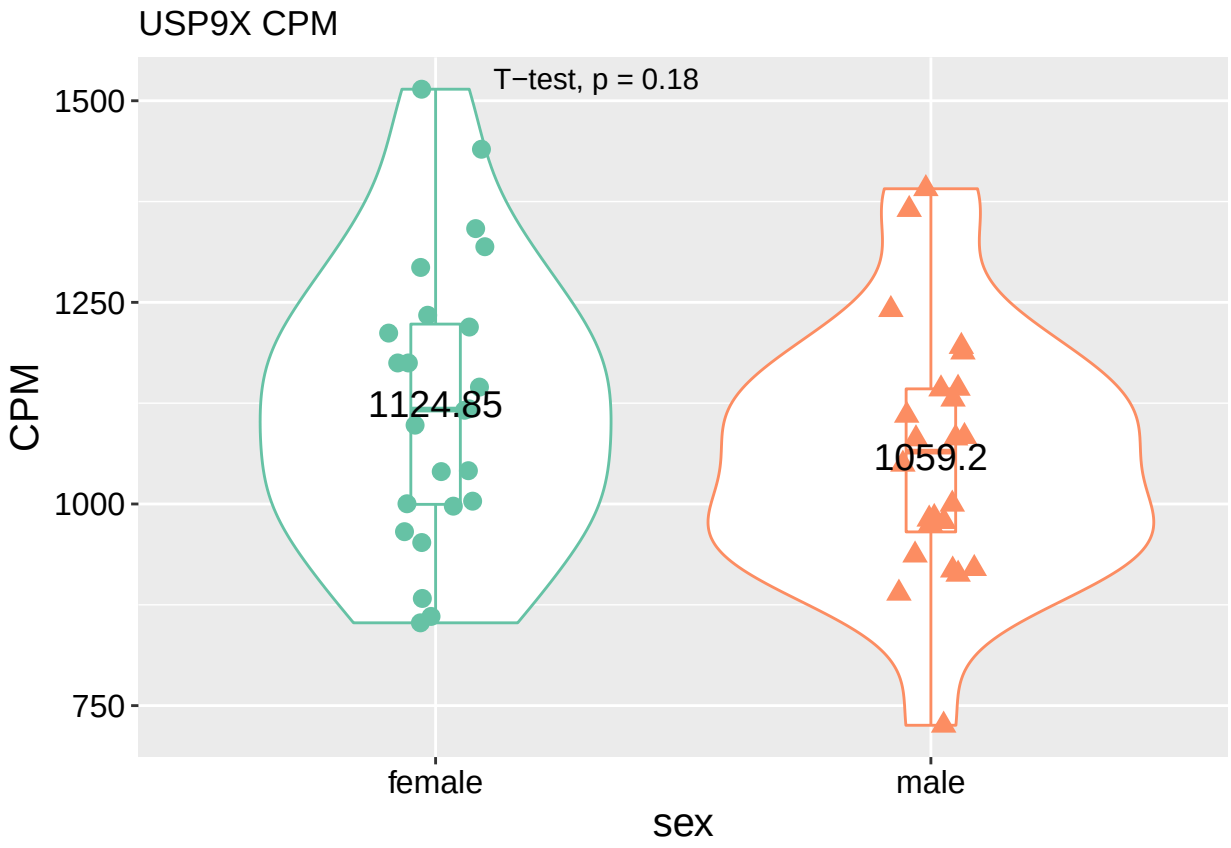


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violinPlots$KDM6A
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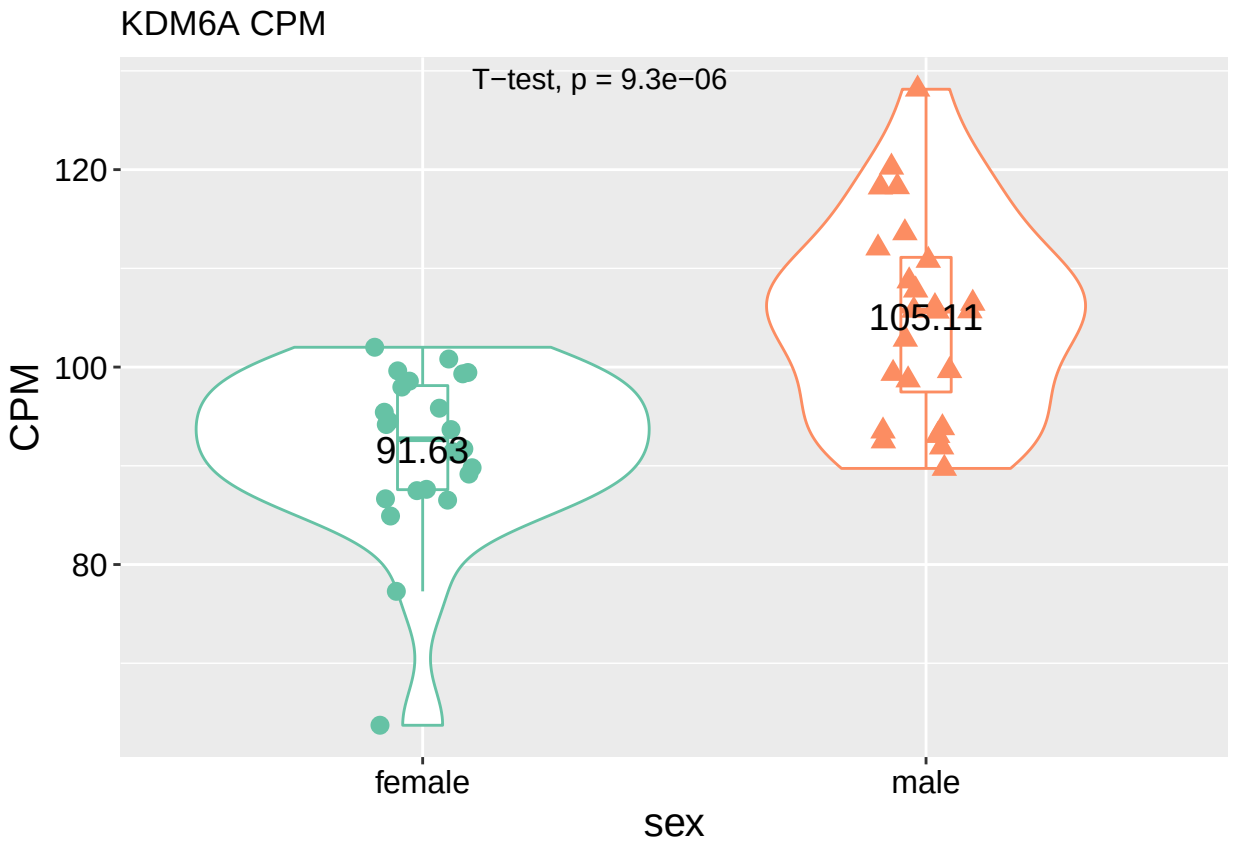


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violinPlots$PRKX
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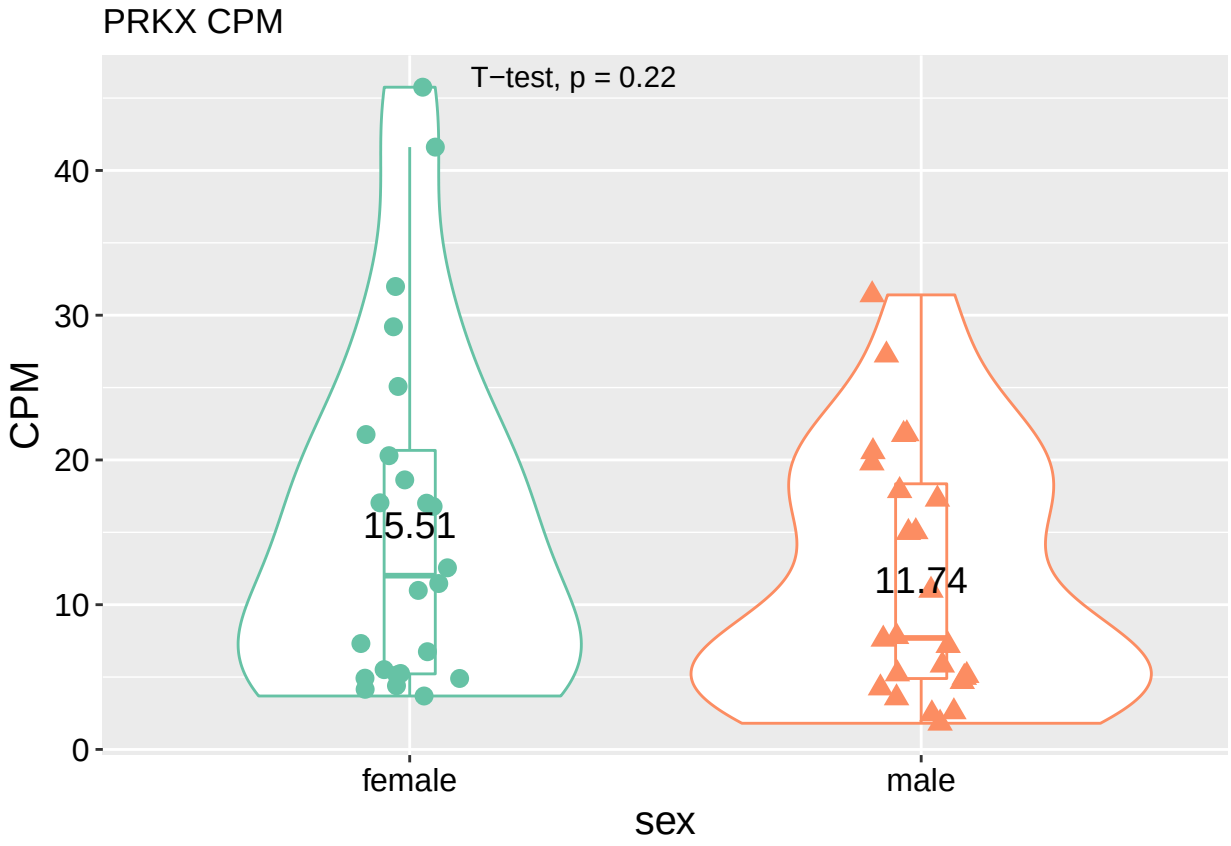


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violinPlots$EIF1AX
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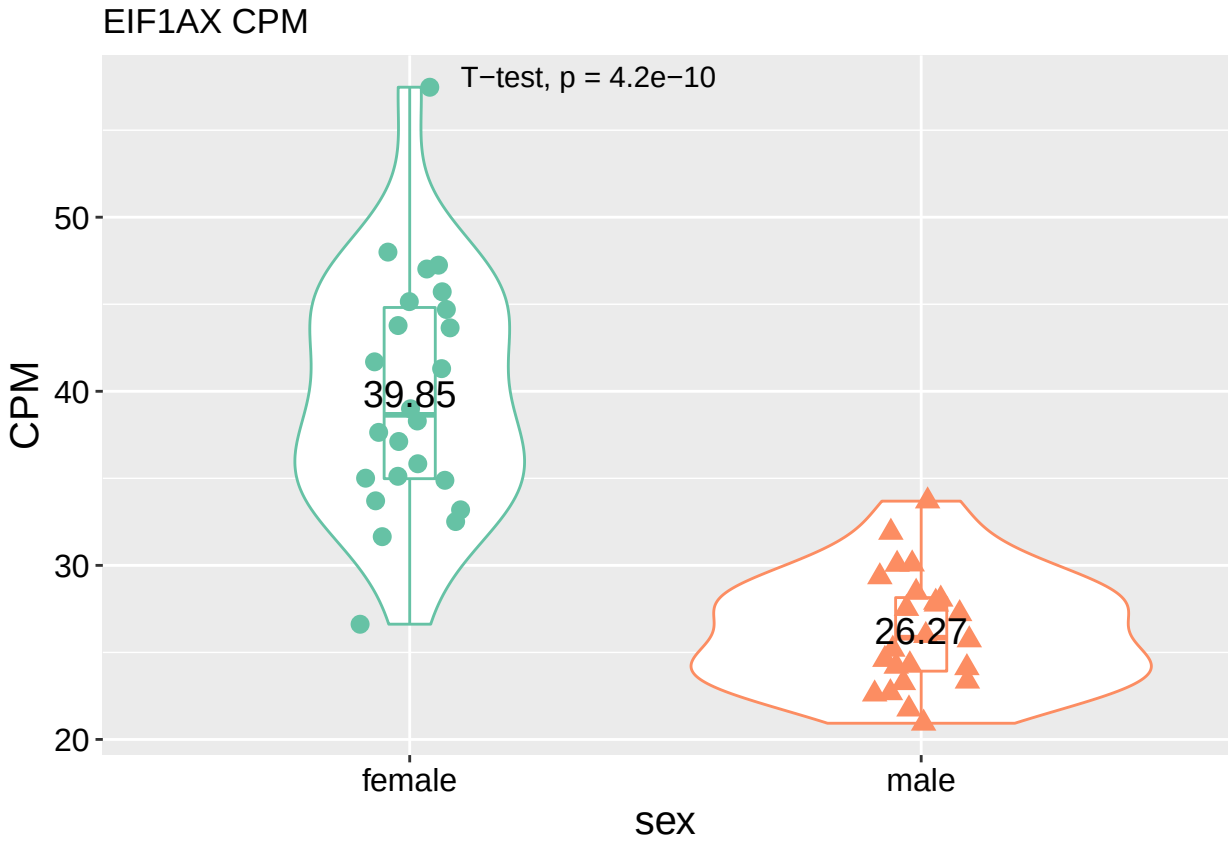
EIF1AX CPM
T−test, p = 4.2e−10
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40
30
20
CPM
39.85
26.27
female
male
sex