

# Analysis of aTSC Mammary RNAseq studies

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## 1 Purpose

To analyse aTSC mammary gland RNAseq dataset

## 2 Experimental Details

Prior to this analysis, reads were mapped to GRCm38 CDS via Salmon v 1.3.0 with flags `--gc-bias`, `--validateMappings` and `-l A`. These data were saved into the quant folders.

```
library(tximport) #loads the tximport package, needed to bring in the salmon files
quant.directories <- 'quants'
sample.directories <- list.dirs(quant.directories,
                                recursive=F) #identify directories that contain salmon output
sample.directories <- sample.directories[grepl('NEH', sample.directories)] #only noura samples
salmon.files <- file.path(sample.directories,"quant.sf") #locate quant files for each directory
salmon.files <- salmon.files[file.exists(salmon.files)]

library("tximeta")

WT <- c(2,4,6,8,10)
WT.names <- paste('1415-NEH-',WT, sep="")
KO <- c(1,3,5,7,9,11)
KO.names <- paste('1415-NEH-',KO, sep="")

coldata <- data.frame(files=salmon.files,
```

```

        stringsAsFactors = F) %>%
separate(col=files,
        sep='_',
        into=c('Folder','names'),
        remove=F) %>%
mutate(Genotype=case_when(names %in% WT.names~'WT',
        names %in% KO.names~'KO')) %>%
mutate(Genotype = relevel(as.factor(Genotype), ref="WT"))

makeLinkedTxome(indexDir='mouse_index',
        source="Ensembl", organism="Mus musculus",
        release="101", genome="GRCm38.101",
        fasta='Mus_musculus.GRCm38.cds.all.fa.gz',
        gtf='Mus_musculus.GRCm38.101.gtf.gz')

se <- tximeta(coldata) # looks at all transcripts and combines them together
#se.exons <- addExons(se) #to summarize to gene level data (individual exons, not done yet)
gse <- summarizeToGene(se) # the total number of genes 22Kx11 samples

```

These data can be found in /Users/davebrid/Documents/GitHub/TissueSpecificTscKnockouts/RNAseq/MammaryGland Adipocyte Tsc1 Knockout in a set of folders located in quants. This script was most recently updated on Fri Apr 2 12:12:11 2021.

```

library(DESeq2)
dds <- DESeqDataSet(gse, ~Genotype)
dds$Genotype <- relevel(dds$Genotype, ref = "WT")
dds <- DESeq(dds) #normalizing samples to each other
dds.genotype <- results(dds,contrast=c("Genotype","KO","WT")) #this must be column - numerator - denominator

library("org.Mm.eg.db")
dds.genotype$symbol <- mapIds(org.Mm.eg.db,
        keys=rownames(dds.genotype),
        column="SYMBOL",
        keytype="ENSEMBL",
        multiVals="first") #annotated with common gene symbols

```

### 3 Summary of Results

```

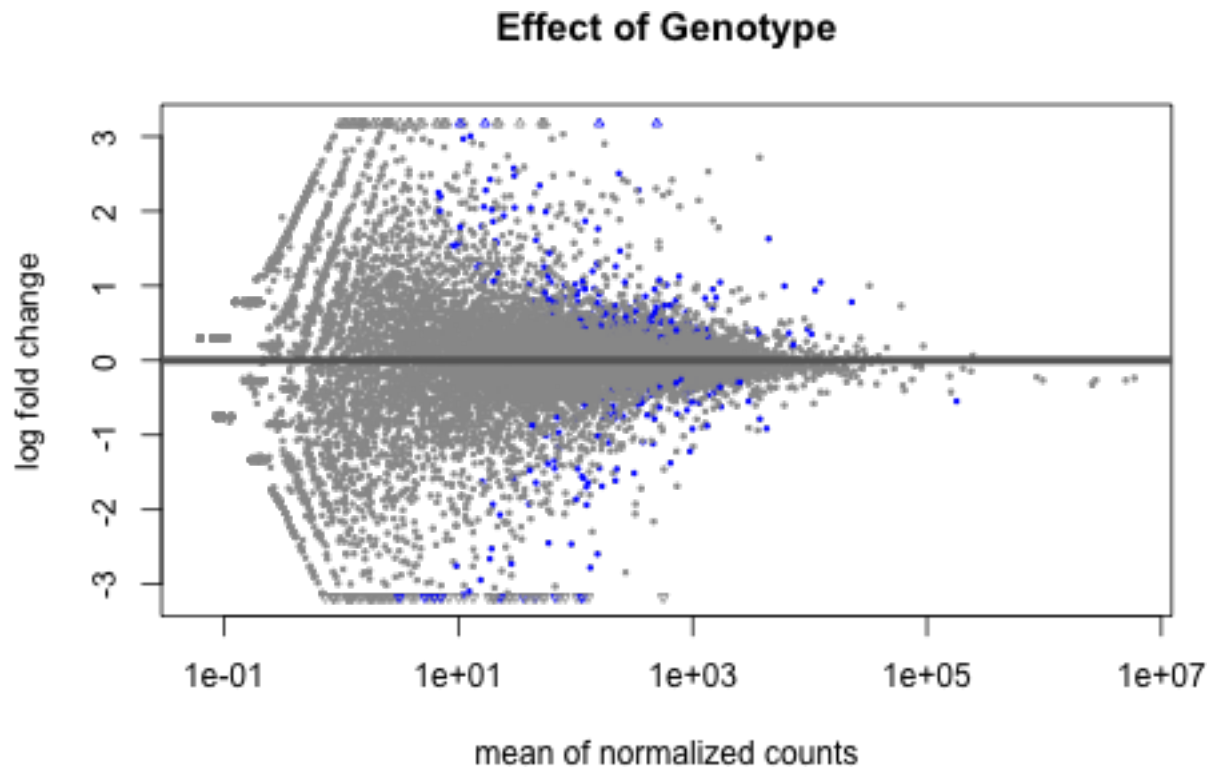
summary(dds.genotype, alpha=0.05)

##
## out of 17605 with nonzero total read count
## adjusted p-value < 0.05
## LFC > 0 (up)      : 85, 0.48%
## LFC < 0 (down)    : 54, 0.31%
## outliers [1]      : 56, 0.32%
## low counts [2]    : 3363, 19%
## (mean count < 3)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results

```

## 4 MA Plot

```
plotMA(dds.genotype, main="Effect of Genotype") #top vs bottom is comparing the 2 genotypes KO vs WT di
```

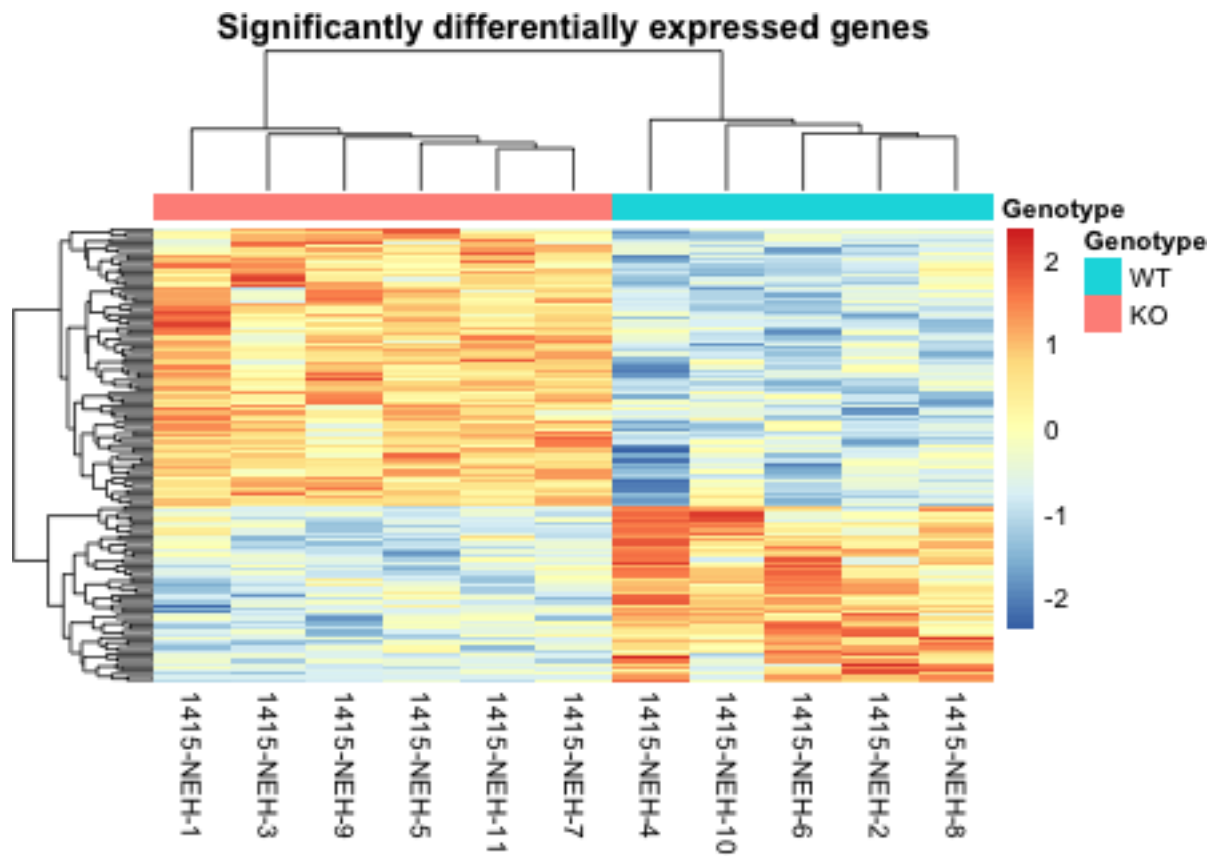


*#x-axis is showing gene abundance , the more the count, the more to the right the dot will be*

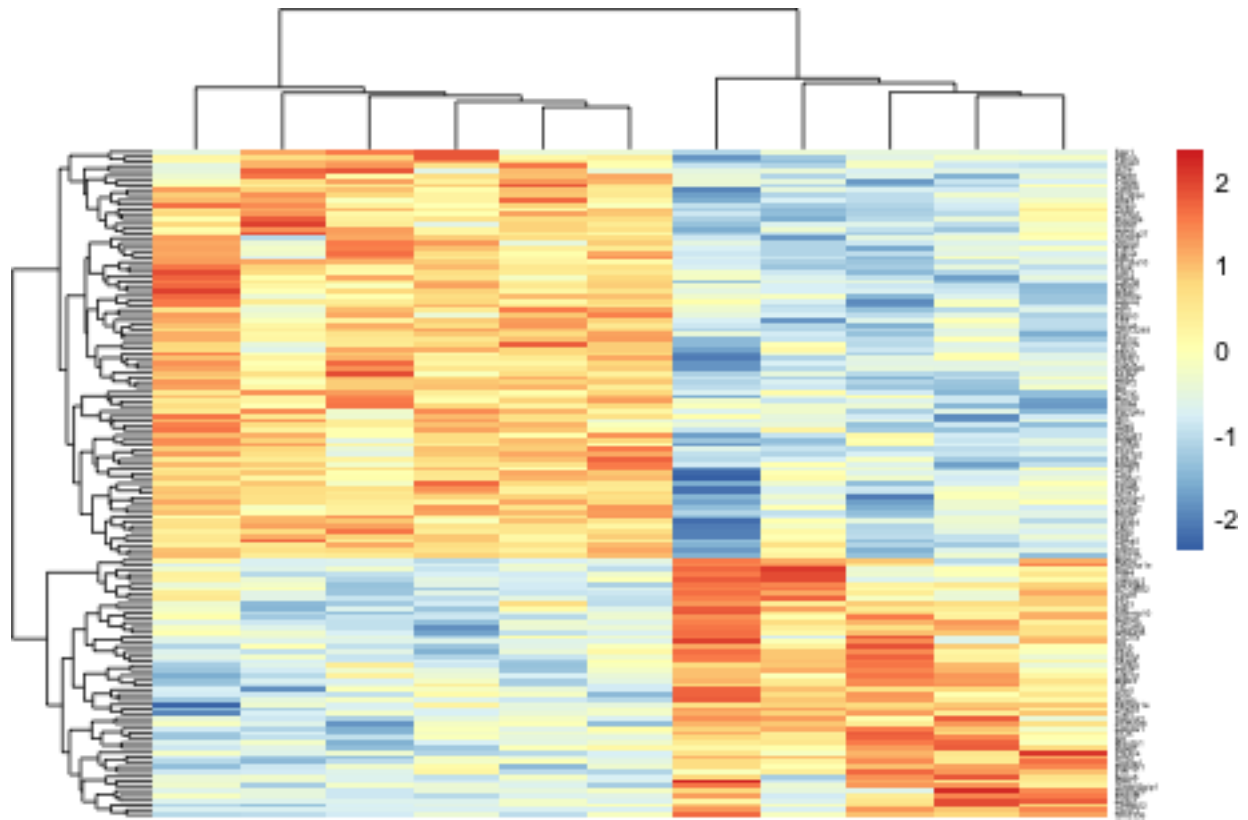
```
library(pheatmap)
ntd <- normTransform(dds)
selected.genes <- subset(dds.genotype, padj<0.05) # pick the 50 most significantly changed genes (up vs
df <- data.frame(colData(dds)$Genotype)
rownames(df) <- colnames(assay(ntd)[rownames(selected.genes),])

name.mapping <- as.data.frame(dds.genotype$symbol)
heatmap.data <- assay(ntd)[rownames(selected.genes),]
rownames(heatmap.data) <- name.mapping[rownames(selected.genes),]

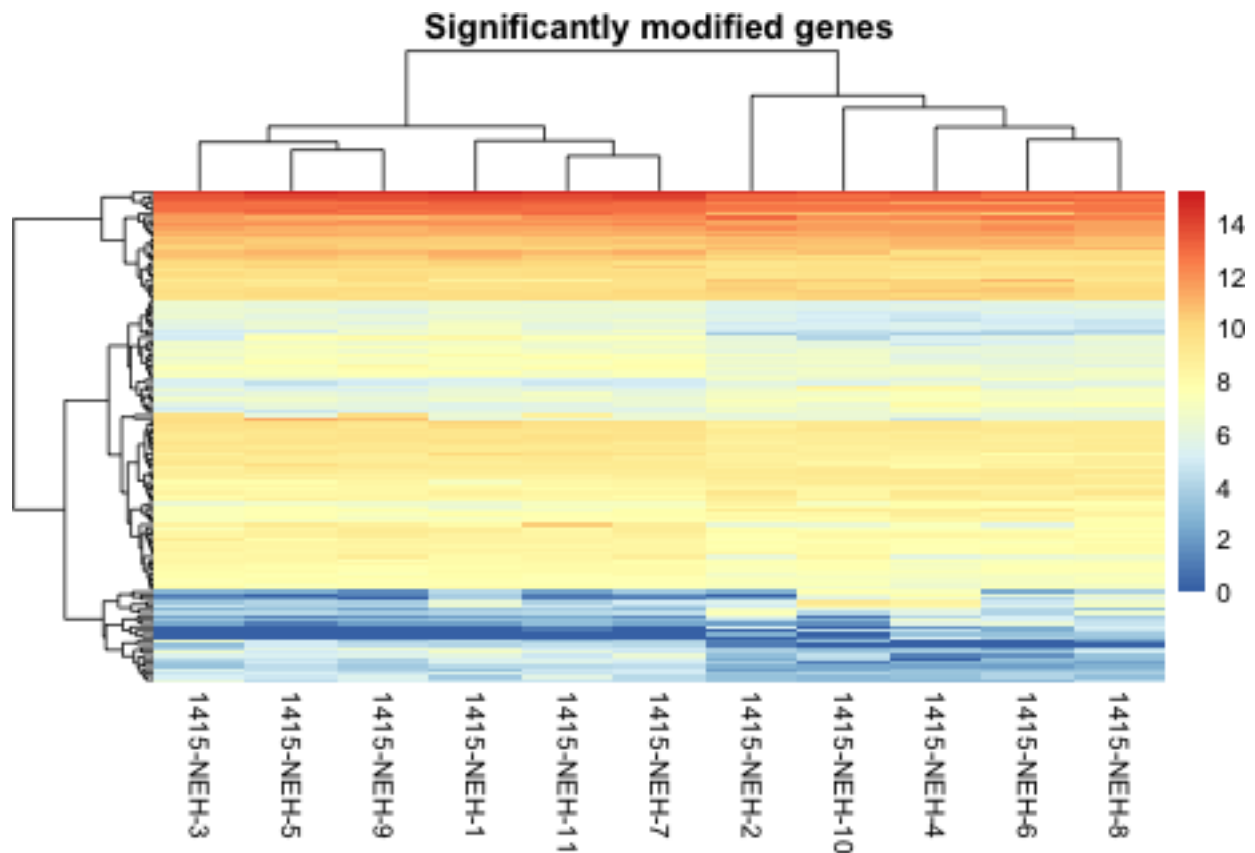
df <- rename(df, 'colData.dds..Genotype'='Genotype')
pheatmap(heatmap.data,
         cluster_rows=T,
         show_rownames = F,
         cluster_cols = T,
         annotation_col=df,
         scale='row',
         main="Significantly differentially expressed genes")
```



```
pheatmap(heatmap.data,
  cluster_rows=T,
  show_rownames = T,
  show_colnames = F,
  fontsize_row = 3,
  cluster_cols = T,
  legend_labels=F,
  border_color=NA,
  scale='row',
  main="")
```



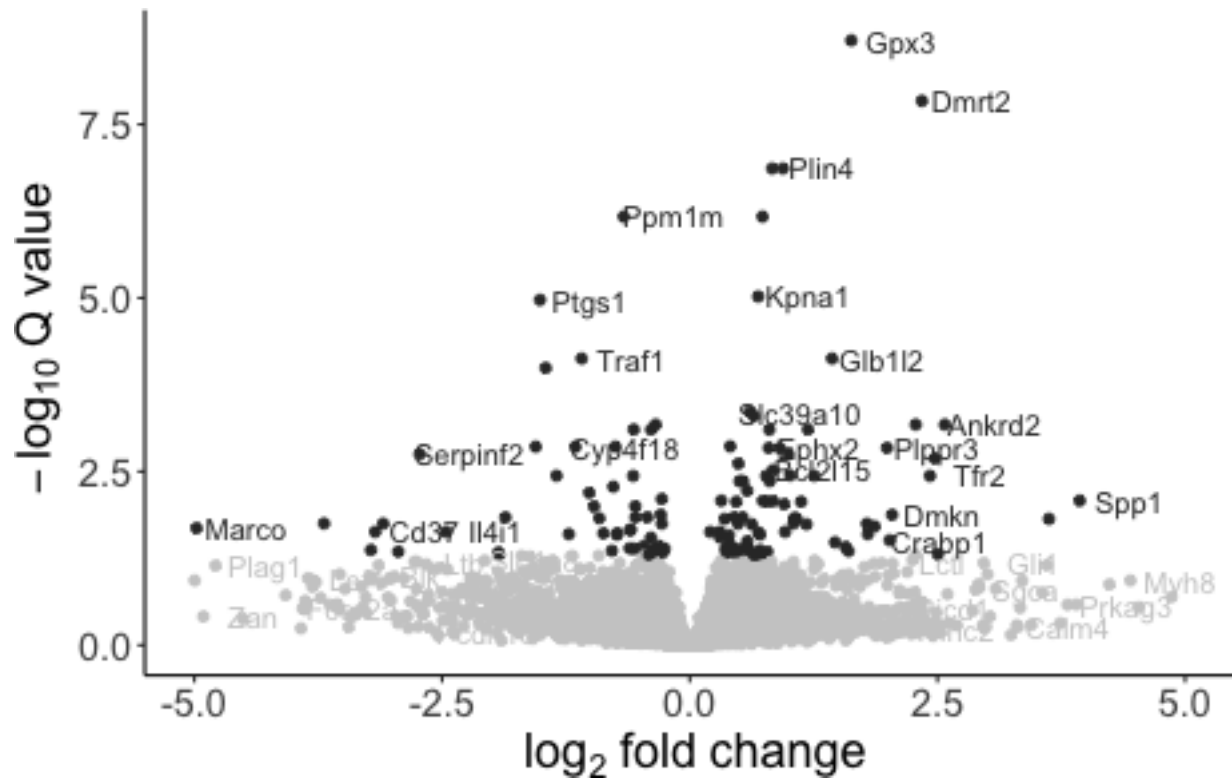
```
pheatmap(heatmap.data,
  cluster_rows=T,
  show_rownames = F,
  cluster_cols = T,
  #annotation_col=df,
  scale='none', #row is each row is the gene with each row having the same average value, normal
  main="Significantly modified genes")
```



```
#from https://www.biostars.org/p/282295/

#par(mar=c(5,5,5,5), cex=1.0, cex.main=1.4, cex.axis=1.4, cex.lab=1.4)

topT <- as.data.frame(dds.genotype)
library(ggplot2)
ggplot(topT, (aes(y=-log10(padj),
                  x=log2FoldChange,
                  col=padj>0.05))) +
  geom_point() +
  labs(y=bquote(~-log[10]~Q~value),
       x=bquote(~log[2]~fold~change),
       subtitle="") +
  geom_text(data=subset(topT, abs(log2FoldChange) > 2 | -log10(padj) > 2.5),
            aes(log2FoldChange, -log10(padj), label=symbol),
            check_overlap = TRUE, nudge_x = 0.5) +
  theme_classic() +
  theme(text=element_text(size=18),
        legend.position="none") +
  scale_colour_grey() +
  xlim(-5,5)
```

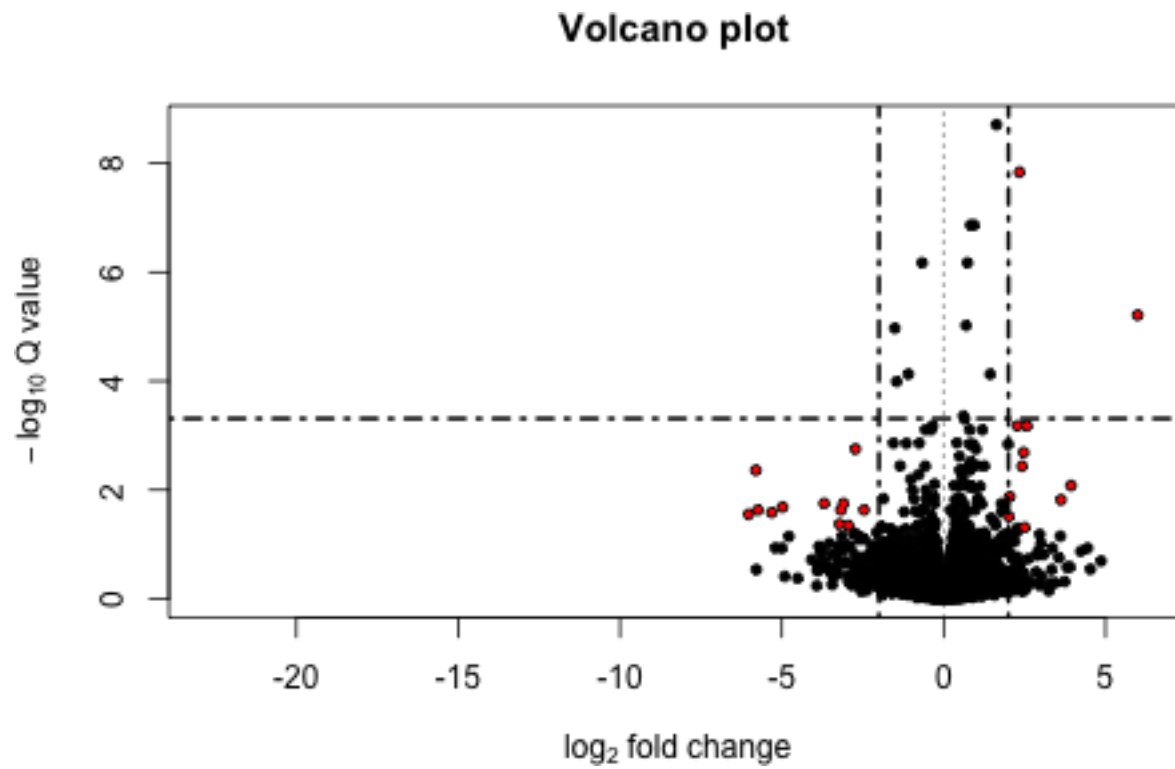


```
#Adjusted P values (FDR Q values)
with(topT, plot(log2FoldChange, -log10(padj), pch=20, main="Volcano plot", cex=1.0, xlab=bquote(-log[2]

with(subset(topT, padj<0.05 & abs(log2FoldChange)>2), points(log2FoldChange, -log10(padj), pch=20, col=

#with(subset(topT, padj<0.05 & abs(log2FoldChange)>2), text(log2FoldChange, -log10(padj), labels=subset

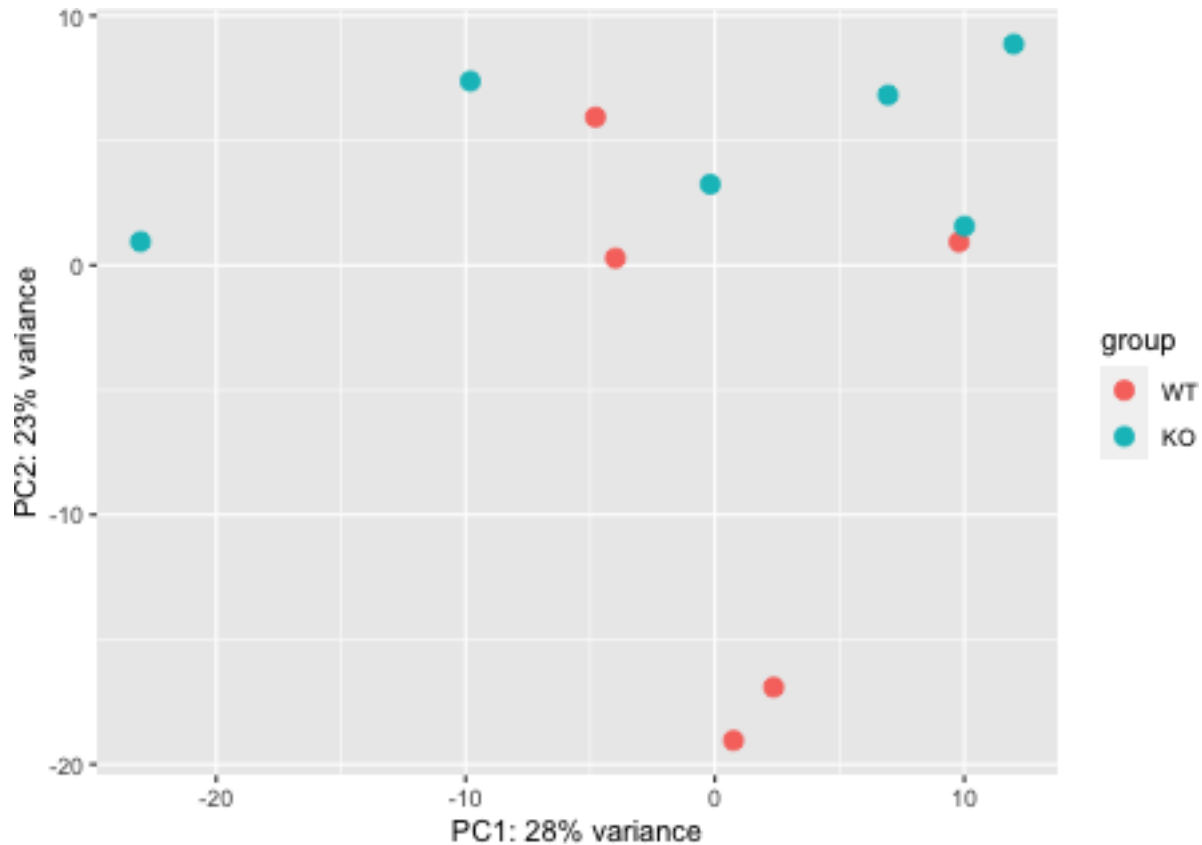
#Add lines for absolute FC>2 and P-value cut-off at FDR Q<0.05
abline(v=0, col="black", lty=3, lwd=1.0)
abline(v=-2, col="black", lty=4, lwd=2.0)
abline(v=2, col="black", lty=4, lwd=2.0)
abline(h=-log10(max(topT$pvalue[topT$padj<0.05], na.rm=TRUE)), col="black", lty=4, lwd=2.0)
```



## 5 Principal Components Analysis

```
vsd <- vst(dds, blind=FALSE)
plotPCA(vsd, intgroup=c("Genotype")) #overall seeing how similar samples are to each other, across samp
```





```
output.file <- 'DESeq2 Results.csv'
output.counts <- 'DESeq2 Normalized Counts.csv'
output.design <- 'Design Table.csv'

write.csv(as.data.frame(dds.genotype), file=output.file)
write.csv(counts(dds, normalized=T), file=output.counts)
write.csv(coldata, file=output.design)
```

## 6 Session Information

```
sessionInfo()
```

```
## R version 4.0.2 (2020-06-22)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS 10.16
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRblas.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.0/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] parallel stats4 stats graphics grDevices utils datasets
```

```

## [8] methods      base
##
## other attached packages:
## [1] ggplot2_3.3.3          pheatmap_1.0.12
## [3] org.Mm.eg.db_3.12.0    AnnotationDbi_1.52.0
## [5] DESeq2_1.30.1          SummarizedExperiment_1.20.0
## [7] Biobase_2.50.0         MatrixGenerics_1.2.1
## [9] matrixStats_0.58.0     GenomicRanges_1.42.0
## [11] GenomeInfoDb_1.26.4    IRanges_2.24.1
## [13] S4Vectors_0.28.1       BiocGenerics_0.36.0
## [15] tximeta_1.8.4          tximport_1.18.0
## [17] dplyr_1.0.5            tidyr_1.1.3
## [19] knitr_1.31
##
## loaded via a namespace (and not attached):
## [1] colorspace_2.0-0        ellipsis_0.3.1
## [3] XVector_0.30.0          farver_2.1.0
## [5] bit64_4.0.5             interactiveDisplayBase_1.28.0
## [7] fansi_0.4.2             xml2_1.3.2
## [9] splines_4.0.2           cachem_1.0.4
## [11] geneplotter_1.68.0      jsonlite_1.7.2
## [13] Rsamtools_2.6.0         annotate_1.68.0
## [15] dbplyr_2.1.0            shiny_1.6.0
## [17] BiocManager_1.30.12     readr_1.4.0
## [19] compiler_4.0.2          httr_1.4.2
## [21] assertthat_0.2.1        Matrix_1.3-2
## [23] fastmap_1.1.0           lazyeval_0.2.2
## [25] later_1.1.0.1           htmltools_0.5.1.1
## [27] prettyunits_1.1.1       tools_4.0.2
## [29] gtable_0.3.0            glue_1.4.2
## [31] GenomeInfoDbData_1.2.4  rappdirs_0.3.3
## [33] Rcpp_1.0.6              vctrs_0.3.7
## [35] Biostrings_2.58.0        rtracklayer_1.50.0
## [37] xfun_0.22               stringr_1.4.0
## [39] mime_0.10               lifecycle_1.0.0
## [41] ensemblDb_2.14.0        XML_3.99-0.6
## [43] AnnotationHub_2.22.0    zlibbioc_1.36.0
## [45] scales_1.1.1            hms_1.0.0
## [47] promises_1.2.0.1        ProtGenerics_1.22.0
## [49] AnnotationFilter_1.14.0 RColorBrewer_1.1-2
## [51] yaml_2.2.1              curl_4.3
## [53] memoise_2.0.0           biomaRt_2.46.3
## [55] stringi_1.5.3           RSQLite_2.2.5
## [57] BiocVersion_3.12.0      highr_0.8
## [59] genefilter_1.72.1       GenomicFeatures_1.42.2
## [61] BiocParallel_1.24.1     rlang_0.4.10
## [63] pkgconfig_2.0.3         bitops_1.0-6
## [65] evaluate_0.14           lattice_0.20-41
## [67] purrr_0.3.4             labeling_0.4.2
## [69] GenomicAlignments_1.26.0 bit_4.0.4
## [71] tidyselect_1.1.0        magrittr_2.0.1
## [73] R6_2.5.0                magick_2.7.1
## [75] generics_0.1.0          DelayedArray_0.16.3
## [77] DBI_1.1.1              pillar_1.5.1

```

|                              |                 |
|------------------------------|-----------------|
| ## [79] withr_2.4.1          | survival_3.2-10 |
| ## [81] Rcurl_1.98-1.3       | tibble_3.1.0    |
| ## [83] crayon_1.4.1         | utf8_1.2.1      |
| ## [85] BiocFileCache_1.14.0 | rmarkdown_2.7   |
| ## [87] progress_1.2.2       | locfit_1.5-9.4  |
| ## [89] grid_4.0.2           | blob_1.2.1      |
| ## [91] digest_0.6.27        | xtable_1.8-4    |
| ## [93] httpuv_1.5.5         | openssl_1.4.3   |
| ## [95] munsell_0.5.0        | askpass_1.1     |