Wilms tumor, SRP358696 bulk RNAseq analysis

Code

Preliminary analysis of the SRP358696 dataset, which contain Wilms tumor samples.

Hide

```
set.seed(123)

library(DESeq2)
library(fgsea)
library(ggplot2)
library(ggrepel)
library(RColorBrewer)
library(patchwork)
library(ggplotify)
library(EnhancedVolcano)
library(ggvenn)
source("/storage/research/dbmr_rubin_lab/scripts/save_png_pdf.R")
source("/storage/research/dbmr_rubin_lab/scripts/fgsea_save_res.R")

plotdir=("plots/")
if(!dir.exists(plotdir)){dir.create(plotdir,  recursive = T)}
```

Load sample names

Hide

```
samples <- scan("../samples.txt", character())
```

Load rsem counts

Hide

```
rsem_res_coding <- read.table("../rsem/all.genes.expected_count.results_coding", header = T, sep = "\t", r
ow.names=1, check.names = F)

rsem_res_coding$gene_name_uniq <- make.unique(rsem_res_coding$gene_name)
```

remove metadata cols (note new rsem has more cols) last col is gene_name_uniq

Hide

```
counts <- rsem_res_coding[c(-1:-27, -ncol(rsem_res_coding))]

rownames(counts) <- rsem_res_coding$gene_name_uniq
```

counts need to be integers

Hide

```
counts <- round(counts)
```

remove low counts

Hide

```
counts <- counts[rowSums(counts) > 5,]
```

Load metadatra

Hide

```
meta <- openxlsx::read.xlsx("../GSE200256_metadata_bulk_RNA_seq.xlsx")

meta$sample <- sub("_", "", meta[["Sample.Name"]])
```

assure same order

Hide

```
identical(meta$sample, colnames(counts))
```

```
[1] TRUE
```

DESeq2

set coldata

Hide

```
coldata <- data.frame(condition = gsub(" .+", "", meta$isolate),
                      tissue = meta$tissue,
                      sex = meta$sex,
                    row.names =  colnames(counts))
```

construct a DESeqDataSet

Hide

```
dds <- DESeqDataSetFromMatrix(countData = counts,
                              colData = coldata,
                              design = ~ condition + tissue + sex)
#relevel
relevel(dds$condition, ref = "Favorable")
```

run DGE inference

Hide

```
dds <- DESeq(dds)
```

Variance stabilizing transformation

Hide

```
vsd <- vst(dds, blind=FALSE)
```

z-scores of vst data (for visualisation etc.)

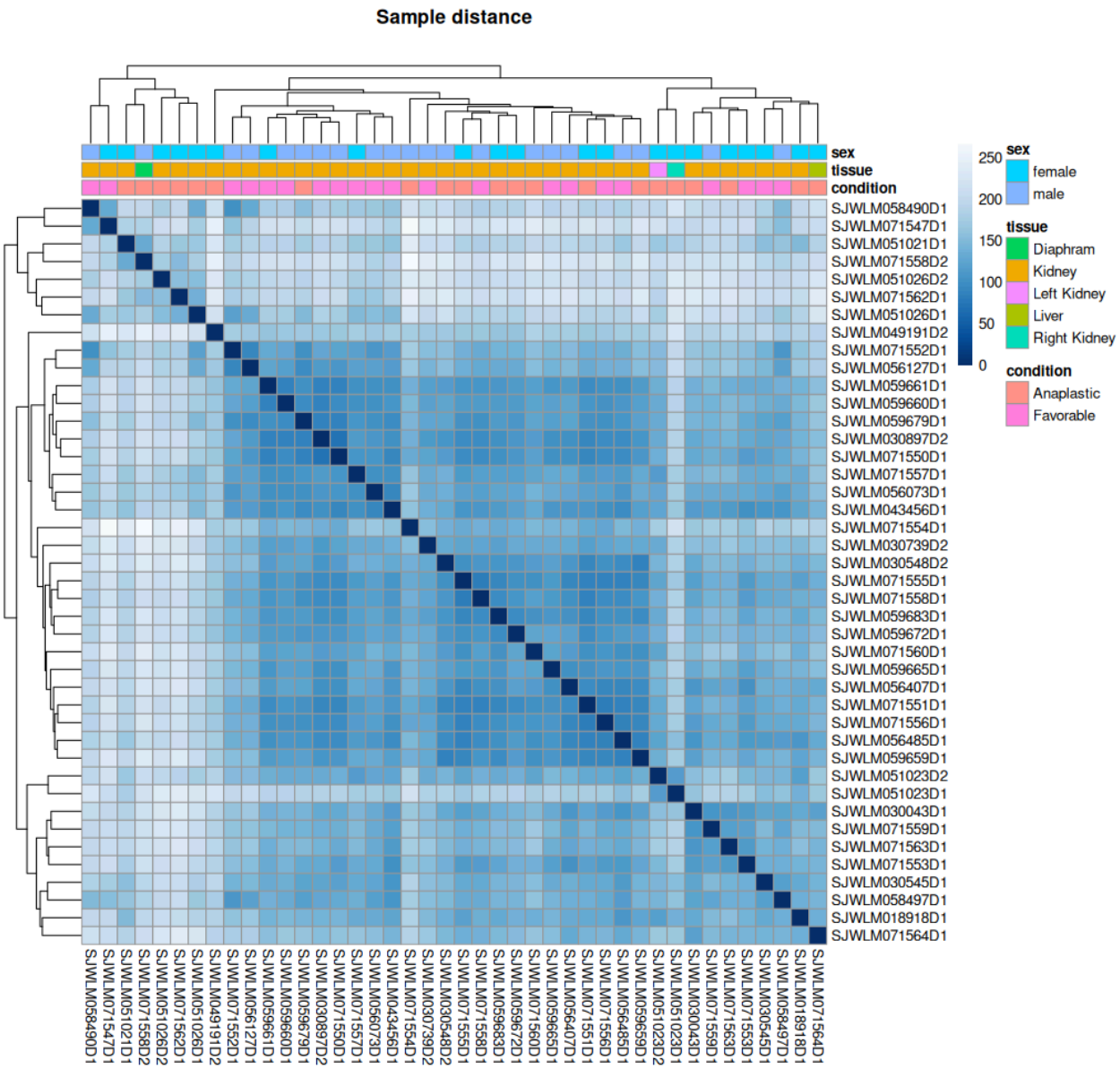
Hide

```
vsd.zscore <- as.data.frame(t(scale(t(assay(vsd)), scale=TRUE, center=TRUE)))
```

Heatmap of the sample-to-sample distances

Hide

```
sampleDists <- dist(t(assay(vsd)))
sampleDistMatrix <- as.matrix(sampleDists)

colors <- colorRampPalette(rev(brewer.pal(9, "Blues")) )(255)

# use fixed cell sizes to look symmetrical, then adjust figure size to have proper margins
p <- pheatmap::pheatmap(sampleDistMatrix,
        clustering_distance_rows=sampleDists,
        clustering_distance_cols=sampleDists,
        col=colors,
        annotation = coldata,
        main = "Sample distance\n",
        cellwidth = 12, cellheight = 12,
        silent = T)

save_png_pdf(p, paste0(plotdir, "heatmap"), height = 12, width = 12)
```

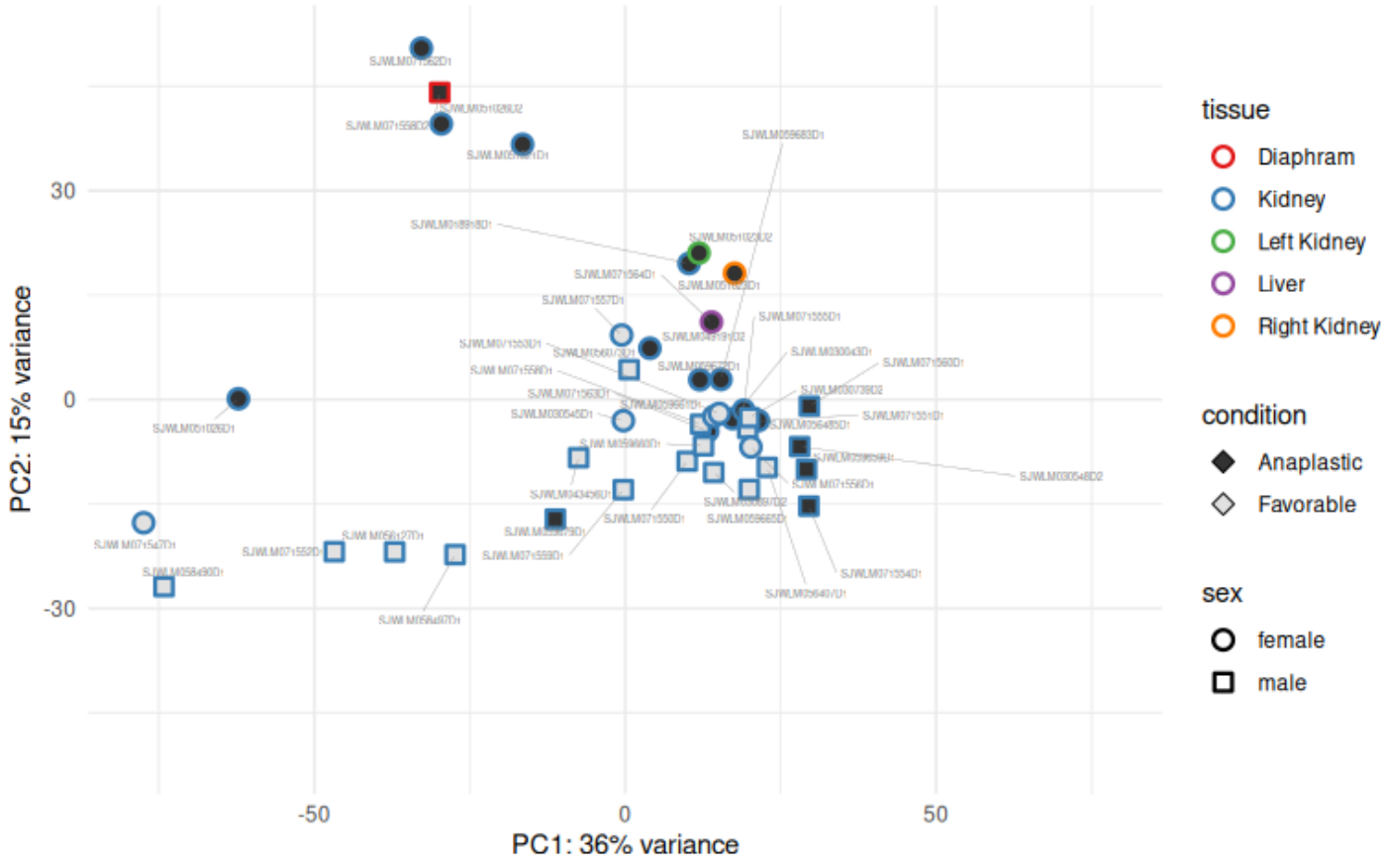

Sample distance
sex
tissue
condition
SJWLM058490D1
SJWLM071547D1
SJWLM051021D1
SJWLM071558D2
SJWLM051026D2
SJWLM071562D1
SJWLM051026D1
SJWLM049191D2
SJWLM071552D1
SJWLM056127D1
SJWLM059661D1
SJWLM059660D1
SJWLM059679D1
SJWLM030897D2
SJWLM071550D1
SJWLM071557D1
SJWLM056073D1
SJWLM043456D1
SJWLM071554D1
SJWLM030739D2
SJWLM030548D2
SJWLM071555D1
SJWLM071558D1
SJWLM059683D1
SJWLM059672D1
SJWLM071560D1
SJWLM059665D1
SJWLM056407D1
SJWLM071551D1
SJWLM071556D1
SJWLM056485D1
SJWLM059659D1
SJWLM051023D2
SJWLM051023D1
SJWLM030043D1
SJWLM071559D1
SJWLM071563D1
SJWLM071553D1
SJWLM030545D1
SJWLM058497D1
SJWLM018918D1
SJWLM071564D1
250
200
150
100
50
0
sex
female
male
tissue
Diaphram
Kidney
Left Kidney
Liver
Right Kidney
condition
Anaplastic
Favorable

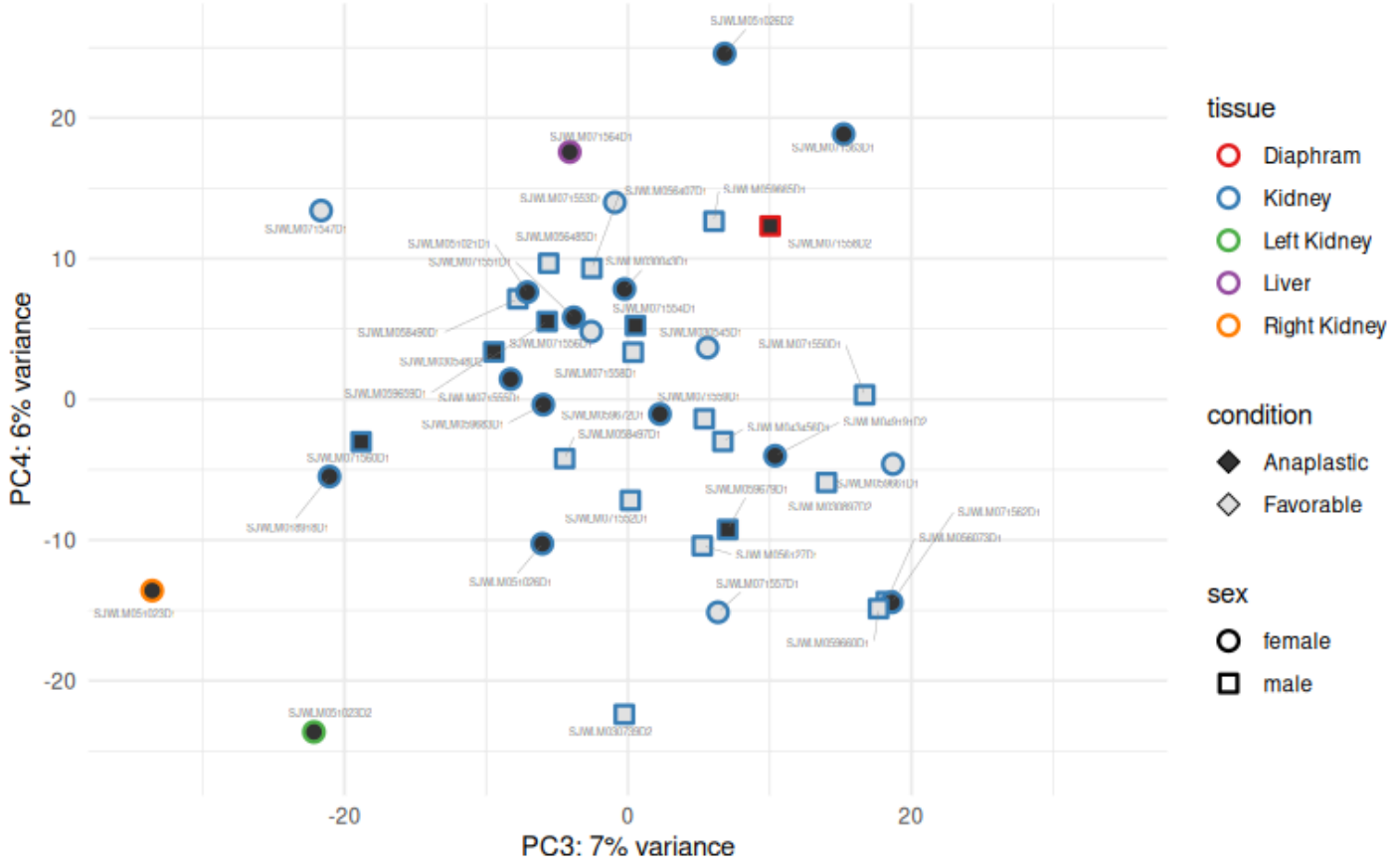
PCA

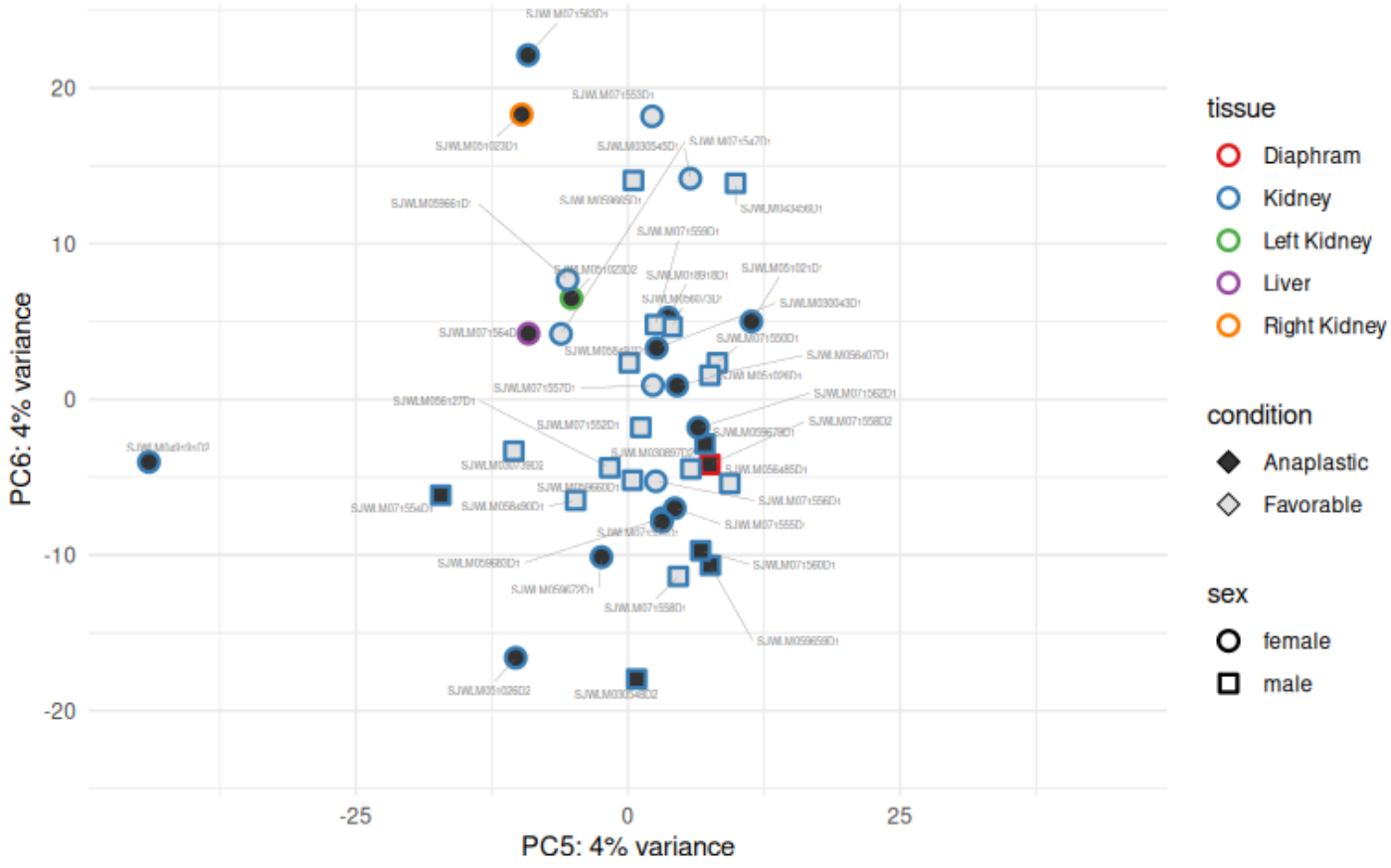
Hide

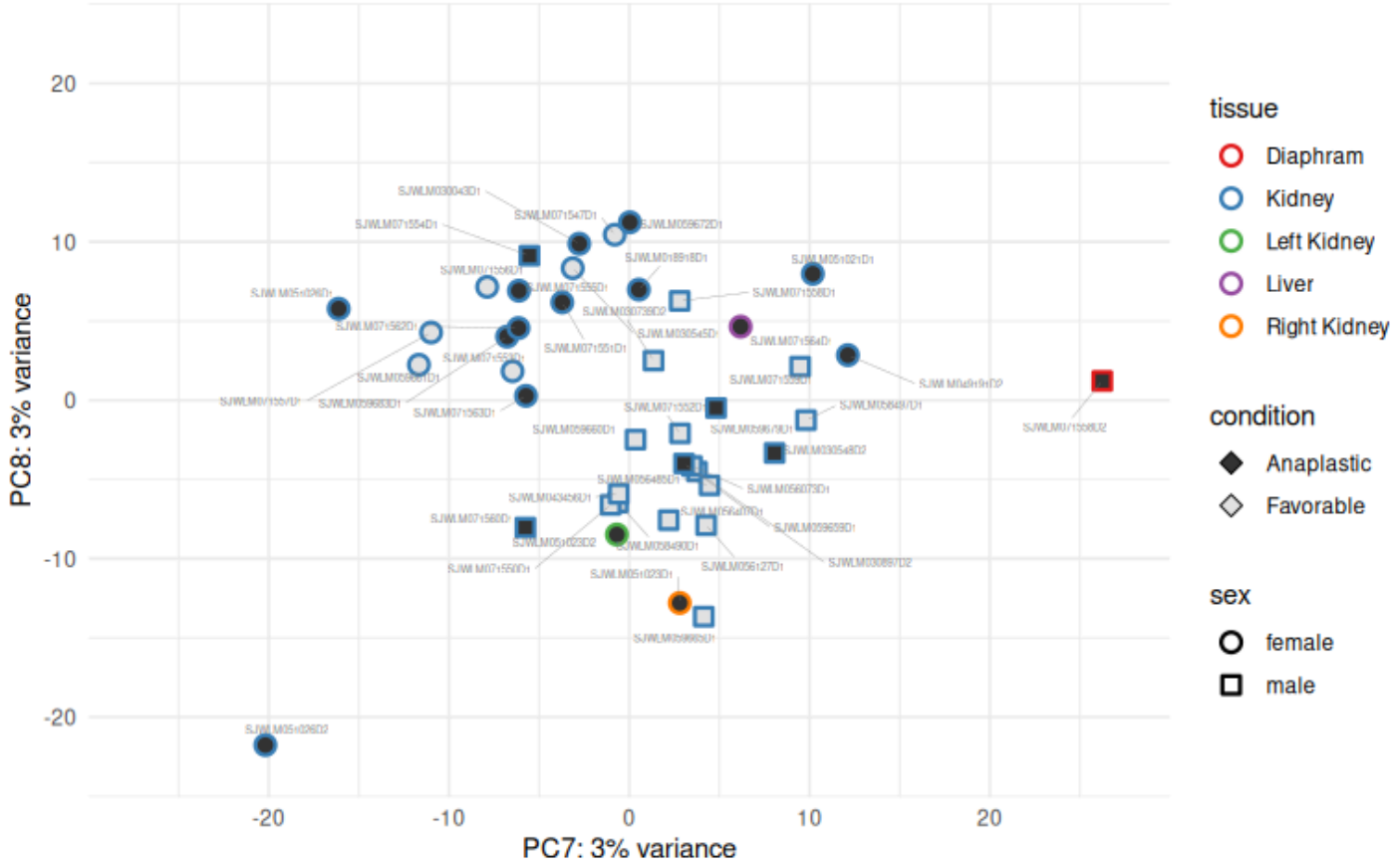
```
pca <- list("PC1-PC2" = 1:2,
            "PC3-PC4" = 3:4,
            "PC5-PC6" = 5:6,
            "PC7-PC8" = 7:8,
            "PC9-PC10" = 9:10)

for (x in names(pca)) {
    cat(x, "\n")
    pcaData <- plotPCA(vsd, intgroup=c("condition"), returnData=TRUE, pcsToUse = pca[[x]])
    percentVar <- round(100 * attr(pcaData, "percentVar"))

    p <- ggplot(pcaData, aes(!!sym(paste0("PC", pca[[x]][1])), !!sym(paste0("PC", pca[[x]][2])), fill=cond
ition, color=tissue, shape=sex)) +
        geom_point(size=3, stroke = 1.2) +
        scale_shape_manual(values = 21:25) + # use fillable shapes
        scale_color_brewer(type = "qual", palette = "Set1",
                           guide = guide_legend(override.aes = list(shape = 1))) + # use a hollow shape in le
gend
        scale_fill_manual(values = c("Anaplastic" = "grey20", "Favorable" = "grey90"),
                           guide = guide_legend(override.aes = list(shape = 23, stroke=0.5))) + # use fillabl
e shape in legend
        xlab(paste0("PC",pca[[x]][1],": ",percentVar[1],"% variance")) +
        ylab(paste0("PC",pca[[x]][2],": ",percentVar[2],"% variance")) +
        xlim(c(-max(abs(pcaData[[paste0("PC", pca[[x]][1])]]))-1, max(abs(pcaData[[paste0("PC", pca[[x]]
[1])]]))+1)) + # PC can be small that dots get trimmed. Expand a bit.
        ylim(c(-max(abs(pcaData[[paste0("PC", pca[[x]][2])]]))-1, max(abs(pcaData[[paste0("PC", pca[[x]]
[2])]]))+1)) + # PC can be small that dots get trimmed. Expand a bit.
        geom_text_repel(aes(label = name),
                           size = 1.5,
                           segment.size = 0.1,
                           colour = 'grey50',
                           box.padding   = 0.10,
                           segment.color = 'grey50',
                           force = 50,
                           max.overlaps = 30,
                           show.legend = F) +
        theme_minimal()

    save_png_pdf(p, paste0(plotdir, x), height = 5, width = 8)
    print(p)
}
```

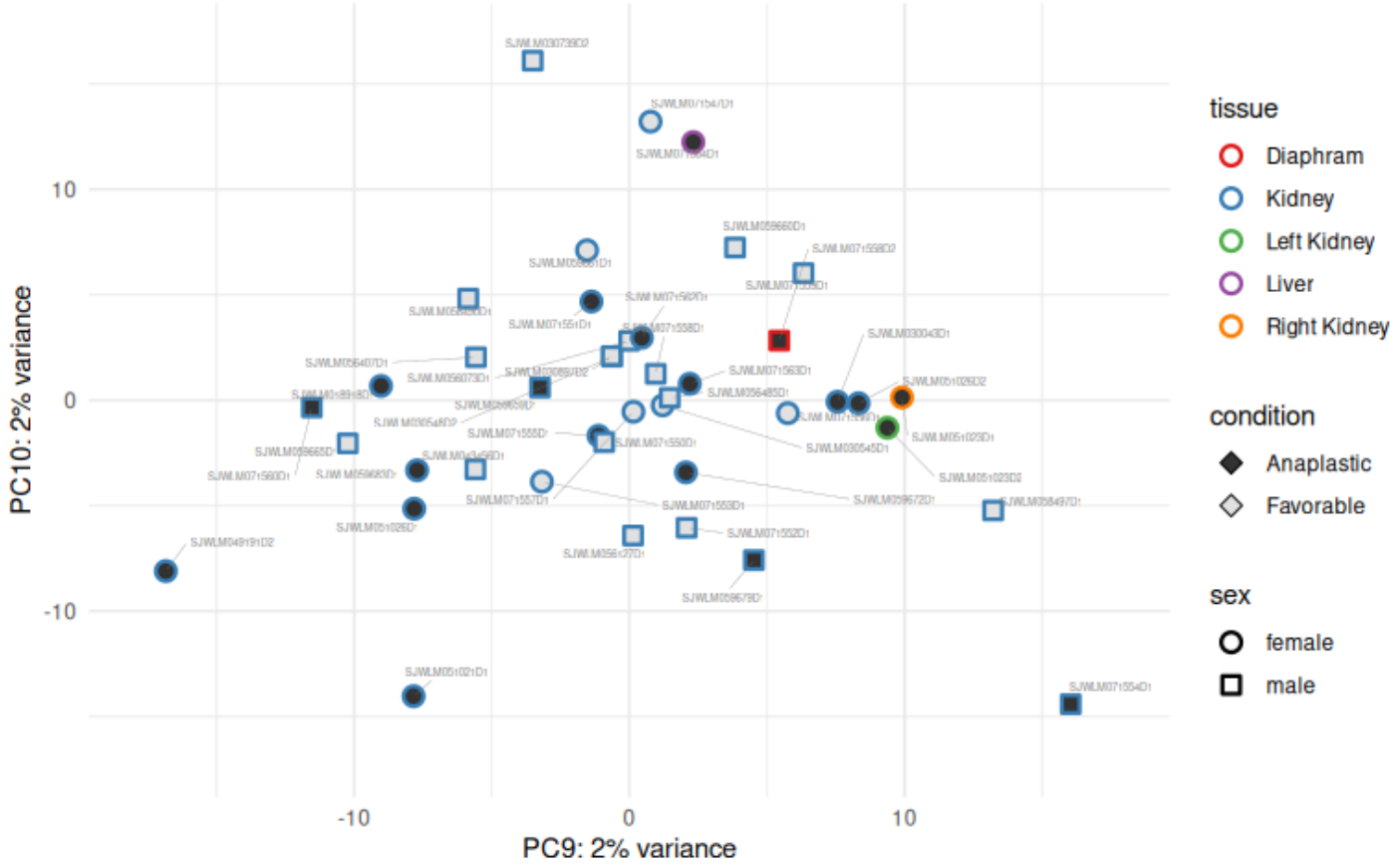
```
PC1-PC2
PC3-PC4
PC5-PC6
PC7-PC8
PC9-PC10
```


PC2: 15% variance
PC1: 36% variance
30
0
-30
-50
0
50
tissue
Diaphram
Kidney
Left Kidney
Liver
Right Kidney
condition
Anaplastic
Favorable
sex
female
male


PC4: 6% variance
PC3: 7% variance
20
10
0
-10
-20
-20
0
20
tissue
Diaphram
Kidney
Left Kidney
Liver
Right Kidney
condition
Anaplastic
Favorable
sex
female
male


PC6: 4% variance
PC5: 4% variance
20
10
0
-10
-20
-25
0
25
tissue
Diaphram
Kidney
Left Kidney
Liver
Right Kidney
condition
Anaplastic
Favorable
sex
female
male


PC8: 3% variance
PC7: 3% variance
20
10
0
-10
-20
-20
-10
0
10
20
tissue
Diaphram
Kidney
Left Kidney
Liver
Right Kidney
condition
Anaplastic
Favorable
sex
female
male



verify comparisons

Hide

```
resultsNames(dds)
```

```
[1] "Intercept"                          "condition_Favorable_vs_Anaplastic" "tissue_Kidney_vs_Diaphram"
[4] "tissue_Left.Kidney_vs_Diaphram"     "tissue_Liver_vs_Diaphram"          "tissue_Right.Kidney_vs_Diaphr
am"
[7] "sex_male_vs_female"
```

Differential expression results for each comparison There is only 1 comparison

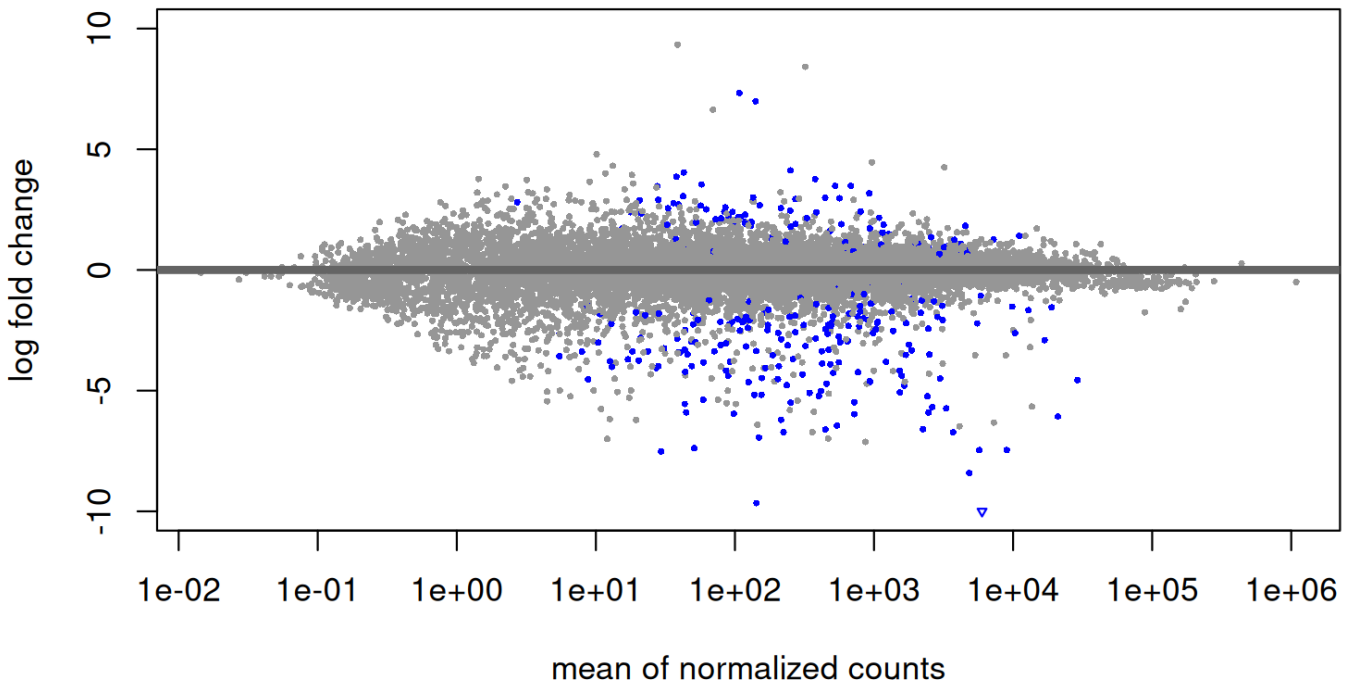
Hide

```
c <- "Anaplastic_vs_Favorable"
res <- results(dds, contrast = c("condition", "Anaplastic", "Favorable"), alpha = 0.05)
```

MA-plot

Hide

```
save_png_pdf(plotMA(res, ylim=c(-10,10)), paste0(plotdir, "DESeq2_res.", c, ".MA-plot"))
```



res to table, and add gene info

Hide

```
res.df <- as.data.frame(res)


res.df <- merge(res.df, rsem_res_coding[,c("gene_name_uniq", "gene_name", "gene_id", "gene_type", "seqname
s", "start", "end", "width", "strand")], by.x=0, by.y="gene_name_uniq", all.x = T, all.y = F, sort = F)
colnames(res.df)[1] <- "gene_name_uniq"
```

save results

Hide

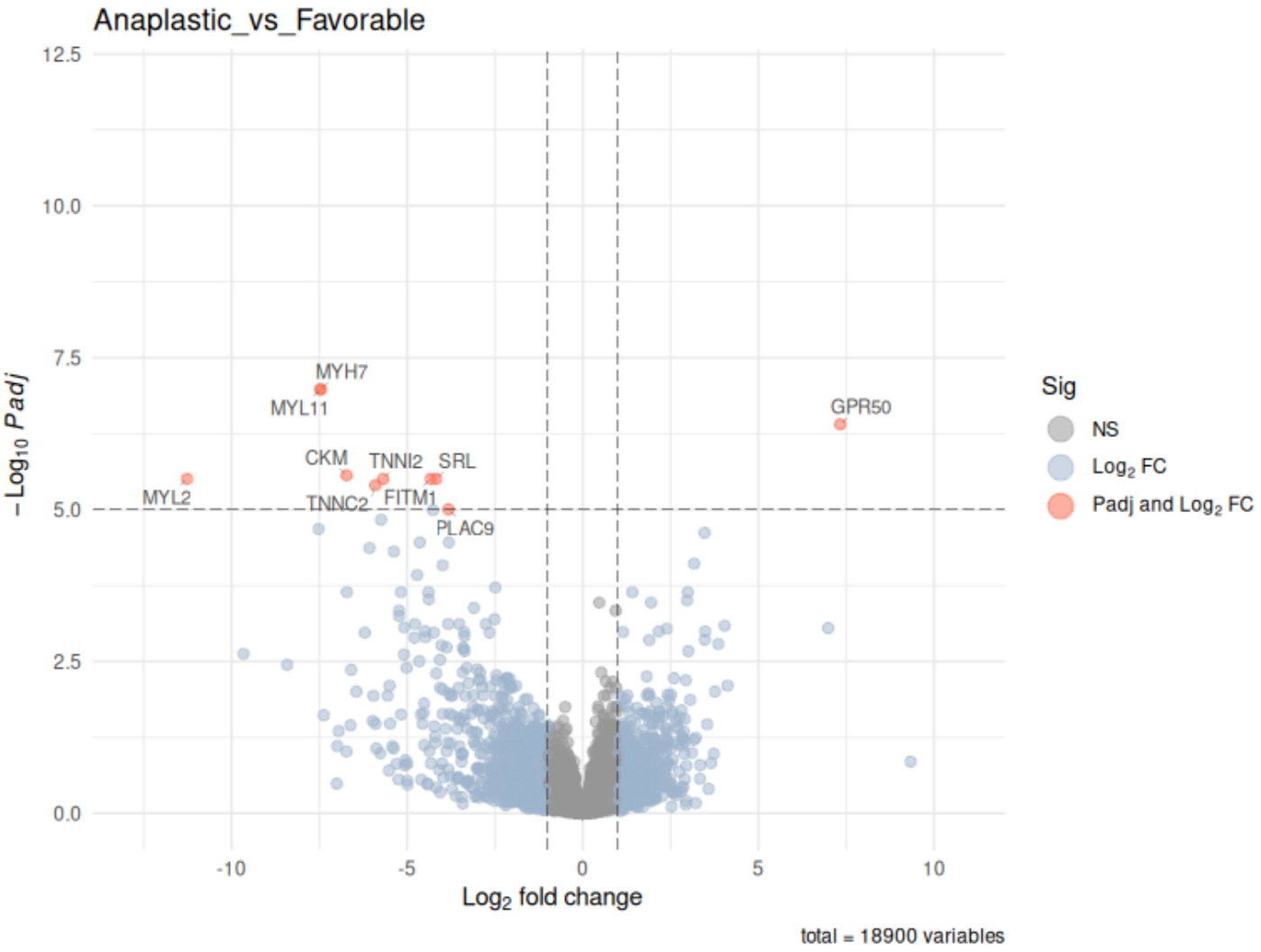
```
openxlsx::write.xlsx(res.df, file="DESeq2_res.xlsx")
```

volcano plots

EnhancedVolcano (https://bioconductor.org/packages/devel/bioc/vignettes/EnhancedVolcano/inst/doc/EnhancedVolcano.html)

Hide

```
p <- EnhancedVolcano(res.df,
                     lab = res.df$gene_name,
                     x = 'log2FoldChange',
                     y = 'padj',
                     ylab = bquote(~-Log[10] ~ italic(Padj)),
                     title = c,
                     subtitle = NULL,
                     cutoffLineCol = "gray10",
                     cutoffLineWidth = 0.2,
                     pointSize = 2,
                     labSize = 3,
                     axisLabSize = 15,
                     titleLabSize = 15,
                     labCol = "gray30",
                     col = c("grey60", "slategray3", "lightpink", "tomato"),
                     legendPosition = 'right',
                     legendLabels=c("NS", expression(Log[2] ~ FC), "Padj", expression(Padj ~ and ~ Log[2] ~
FC)),
                     legendLabSize = 10,
                     max.overlaps = 50,
                     drawConnectors = T,
                     widthConnectors = 0.1,
                     arrowheads = F,
                     colConnectors = "grey30",
                     directionConnectors="both" # 'y' works ok for fewer genes. ideal should be upregulated
to one side, down to the other
                     ) +
    theme_minimal()

save_png_pdf(p, paste0(plotdir, "EnhancedVolcano.", c), height = 6, width = 8)
```



fgsea Msigdb

Load get gene sets in gmt

Hide

```
msigdb.hs.gmt <- readRDS("/storage/research/dbmr_rubin_lab/resources/msigdb/msigdb.hs.gmt.rds")
msigdb.hs.info <- readRDS("/storage/research/dbmr_rubin_lab/resources/msigdb/msigdb.hs.info.df.rds")
```

make rank

Hide

```
res.rank <- setNames(res.df$stat, make.unique(res.df$gene_name))
```

run only relevant collections

Hide

```
collections <- c("C2.CP", "C2.CP:KEGG_LEGACY", "C2.CP:KEGG_MEDICUS", "C2.CP:REACTOME", "C4.3CA", "C4.CGN",
"C4.CM", "C5.GO:BP", "C5.GO:MF", "C6", "C7.IMMUNESIGDB", "C8", "H")
```

run fgsea

Hide

```
res.fgseaRes.msigdb <- sapply(collections, function(x) {
    cat(c, x, "\n")
    fgsea(pathways = msigdb.hs.gmt[[x]],
            stats = res.rank,
            eps = 0.0, minSize = 15, maxSize = 500)
}, simplify=F)

# Save session
save.image(file="session.RData")
```

Plot fgsea results

Hide

```
fgsea_save_res(res.fgseaRes.msigdb,
                rank = res.rank,
                basename = "fgsea.",
                gmt = msigdb.hs.gmt[collections],
                gmt_info = msigdb.hs.info,
                suffix = c,
                subtitle = c)
```

DE on transcript level

load rsem counts

Hide

```
rsem_res_coding.transcript <- read.table("../rsem/all.isoforms.expected_count.results_coding", header = T,
sep = "\t", row.names=1, check.names = F)

# remove metadata cols (note new rsem has more cols)
counts.transcript <- rsem_res_coding.transcript[-1:-27]

# rownames can be transcript_name (they are unique)
sum(duplicated(rsem_res_coding.transcript$transcript_name))
# [1] 0
# but replace "-" with "_" so DESeq2 does not complain
rownames(counts.transcript) <- sub("-", "_", rsem_res_coding.transcript$transcript_name)
```

Prepare counts

Hide

```
# counts.transcript need to be integers
counts.transcript <- round(counts.transcript)

# remove low counts.transcript
counts.transcript <- counts.transcript[rowSums(counts.transcript) > 5,]
```

DESeq2

construct a DESeqDataSet

Hide

```
dds.transcript <- DESeqDataSetFromMatrix(countData = counts.transcript,
                              colData = coldata,
                              design = ~ condition + tissue + sex)

# relevel
relevel(dds.transcript$condition, ref = "Favorable")
```

run DGE inference

Hide

```
dds.transcript <- DESeq(dds.transcript)
```

Variance stabilizing transformation

Hide

```
vsd.transcript <- vst(dds.transcript, blind=FALSE)
```

z-scores of vst data (for visualisation etc.)

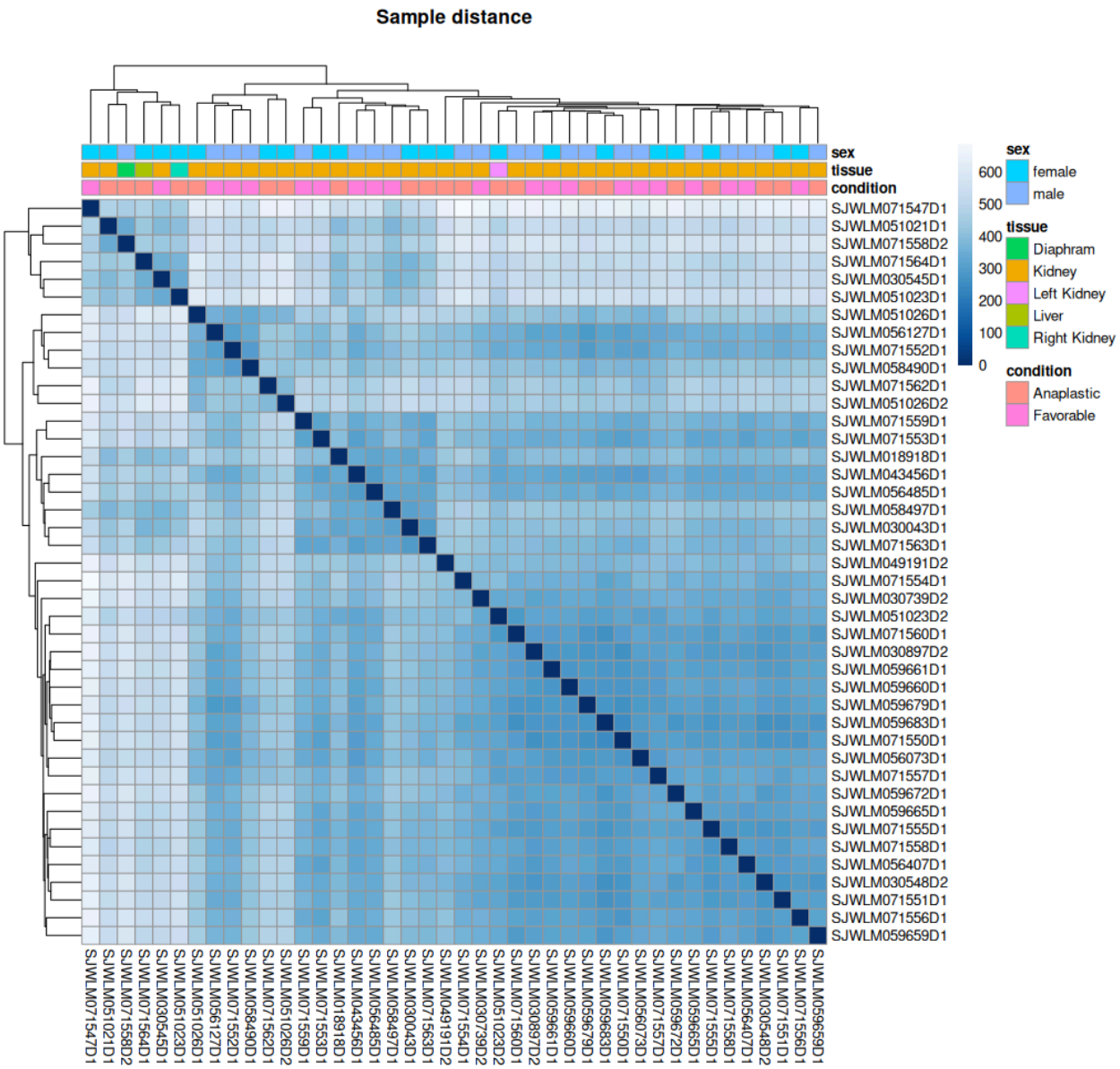
Hide

```
vsd.transcript.zscore <- as.data.frame(t(scale(t(assay(vsd.transcript)), scale=TRUE, center=TRUE)))
```

Heatmap of the sample-to-sample distances

Hide

```
sampleDists <- dist(t(assay(vsd.transcript)))
sampleDistMatrix <- as.matrix(sampleDists)

colors <- colorRampPalette(rev(brewer.pal(9, "Blues")) )(255)

# use fixed cell sizes to look symmetrical, then adjust figure size to have proper margins
p <- pheatmap::pheatmap(sampleDistMatrix,
        clustering_distance_rows=sampleDists,
        clustering_distance_cols=sampleDists,
        col=colors,
        annotation = coldata,
        main = "Sample distance\n",
        cellwidth = 12, cellheight = 12,
        silent=T)

save_png_pdf(p, paste0(plotdir, "heatmap.transcript"), height = 12, width = 12)
```


Sample distance
sex
tissue
condition
SJWLM071547D1
SJWLM051021D1
SJWLM071558D2
SJWLM071564D1
SJWLM030545D1
SJWLM051023D1
SJWLM051026D1
SJWLM056127D1
SJWLM071552D1
SJWLM058490D1
SJWLM071562D1
SJWLM051026D2
SJWLM071559D1
SJWLM071553D1
SJWLM018918D1
SJWLM043456D1
SJWLM056485D1
SJWLM058497D1
SJWLM030043D1
SJWLM071563D1
SJWLM049191D2
SJWLM071554D1
SJWLM030739D2
SJWLM051023D2
SJWLM071560D1
SJWLM030897D2
SJWLM059661D1
SJWLM059660D1
SJWLM059679D1
SJWLM059683D1
SJWLM071550D1
SJWLM056073D1
SJWLM071557D1
SJWLM059672D1
SJWLM059665D1
SJWLM071555D1
SJWLM071558D1
SJWLM056407D1
SJWLM030548D2
SJWLM071551D1
SJWLM071556D1
SJWLM059659D1
600
500
400
300
200
100
0
sex
female
male
tissue
Diaphram
Kidney
Left Kidney
Liver
Right Kidney
condition
Anaplastic
Favorable

PCA

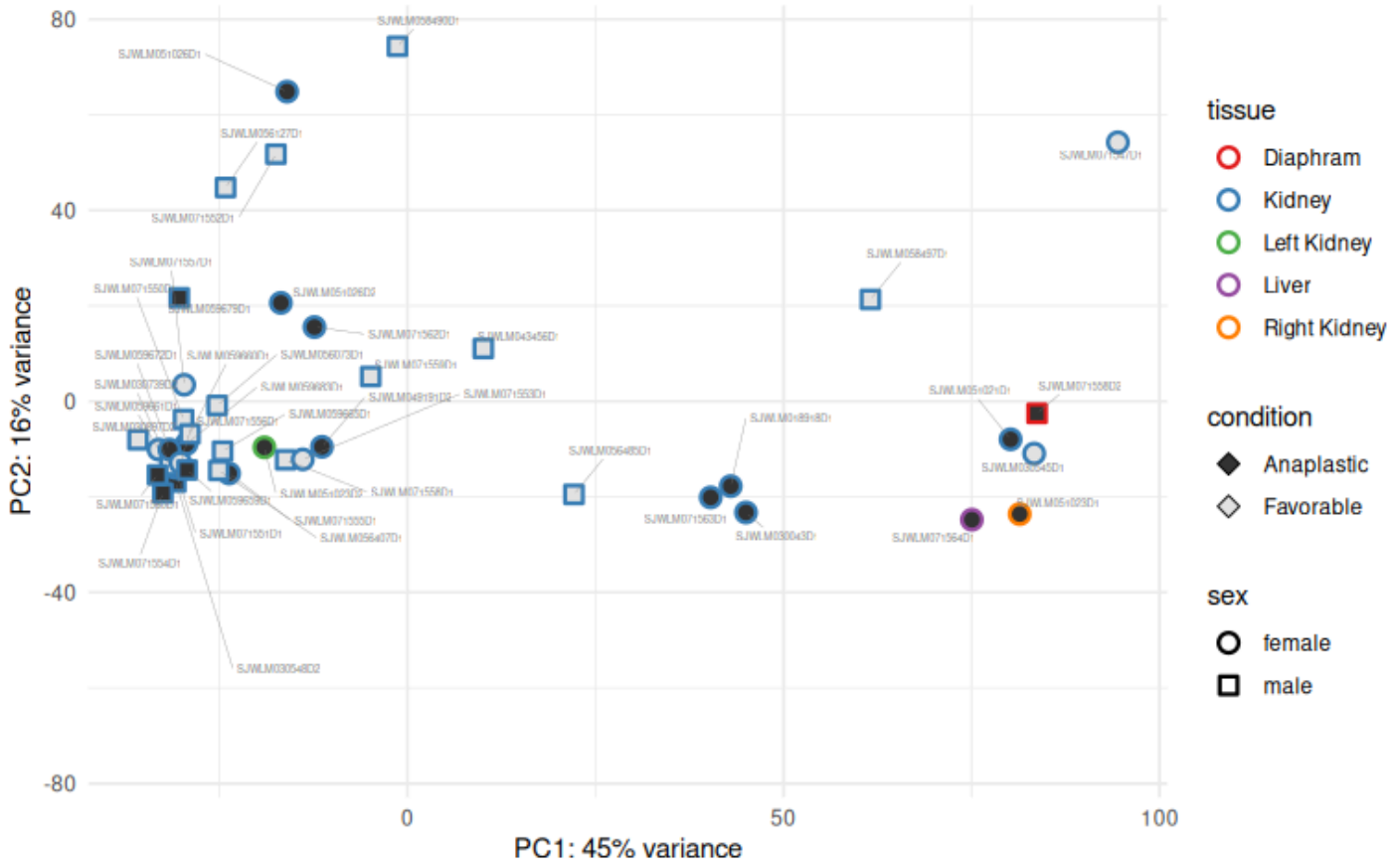
Hide

```
pcaData <- plotPCA(vsd.transcript, intgroup=c("condition"), returnData=TRUE)
percentVar <- round(100 * attr(pcaData, "percentVar"))

p <- ggplot(pcaData, aes(PC1, PC2, fill=condition, color=tissue, shape=sex)) +
    geom_point(size=3, stroke = 1.2) +
    scale_shape_manual(values = 21:25) + # use fillable shapes
    scale_color_brewer(type = "qual", palette = "Set1",
                       guide = guide_legend(override.aes = list(shape = 1))) + # use a hollow shape in legend
    scale_fill_manual(values = c("Anaplastic" = "grey20", "Favorable" = "grey90"),
                       guide = guide_legend(override.aes = list(shape = 23, stroke=0.5))) + # use fillable sh
ape in legend
    xlab(paste0("PC1: ",percentVar[1],"% variance")) +
    ylab(paste0("PC2: ",percentVar[2],"% variance")) +
    ylim(c(-max(abs(pcaData$PC2))-1, max(abs(pcaData$PC2))+1)) + # if PC2 is small dots get trimmed. Expan
d a bit.
    geom_text_repel(aes(label = name),
                       size = 1.5,
                       segment.size = 0.1,
                       colour = 'grey50',
                       box.padding   = 0.10,
                       segment.color = 'grey50',
                       force = 50,
                       max.overlaps = 30,
                       show.legend = F) +
    theme_minimal()

save_png_pdf(p, paste0(plotdir, "PCA.transcript"), height = 5, width = 8)
```

```
null device
          1
```

Hide

```
print(p)
```



Fetch results

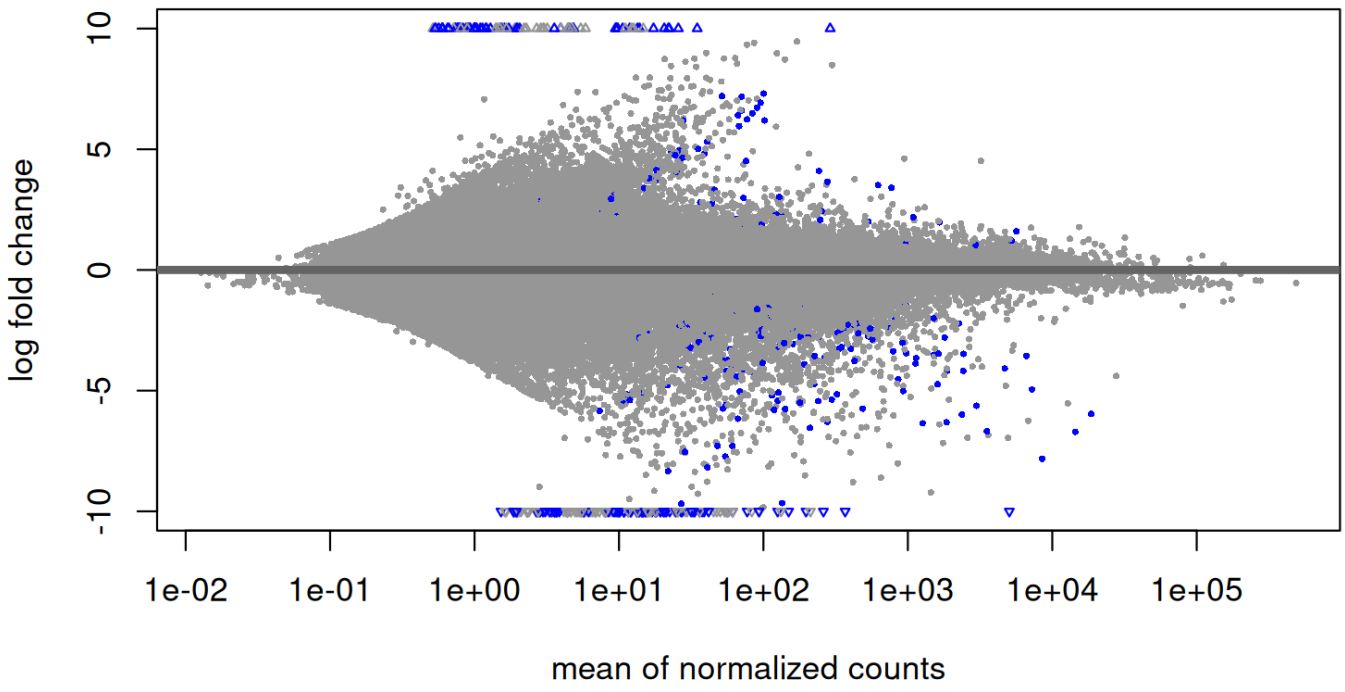
Hide

```
res.transcript <- results(dds.transcript, contrast = c("condition", "Anaplastic", "Favorable"), alpha = 0.
05)
```

MA-plot

Hide

```
save_png_pdf(plotMA(res.transcript, ylim=c(-20,20)), paste0(plotdir, "DESeq2_res.transcript.", c, ".MA-plo
t"))
```



res to table, and add gene info

Hide

```
res.transcript.df <- as.data.frame(res.transcript)

# _ to - so that we can merge
res.transcript.df$transcript_name <- sub("_", "-", rownames(res.transcript))

res.transcript.df <- merge(res.transcript.df, rsem_res_coding.transcript[,c("transcript_name", "transcript
_id", "transcript_type", "gene_name", "gene_id", "seqnames", "start", "end", "width", "strand")], by="tran
script_name", all.x = T, all.y = F, sort = F)
```

save results

Hide

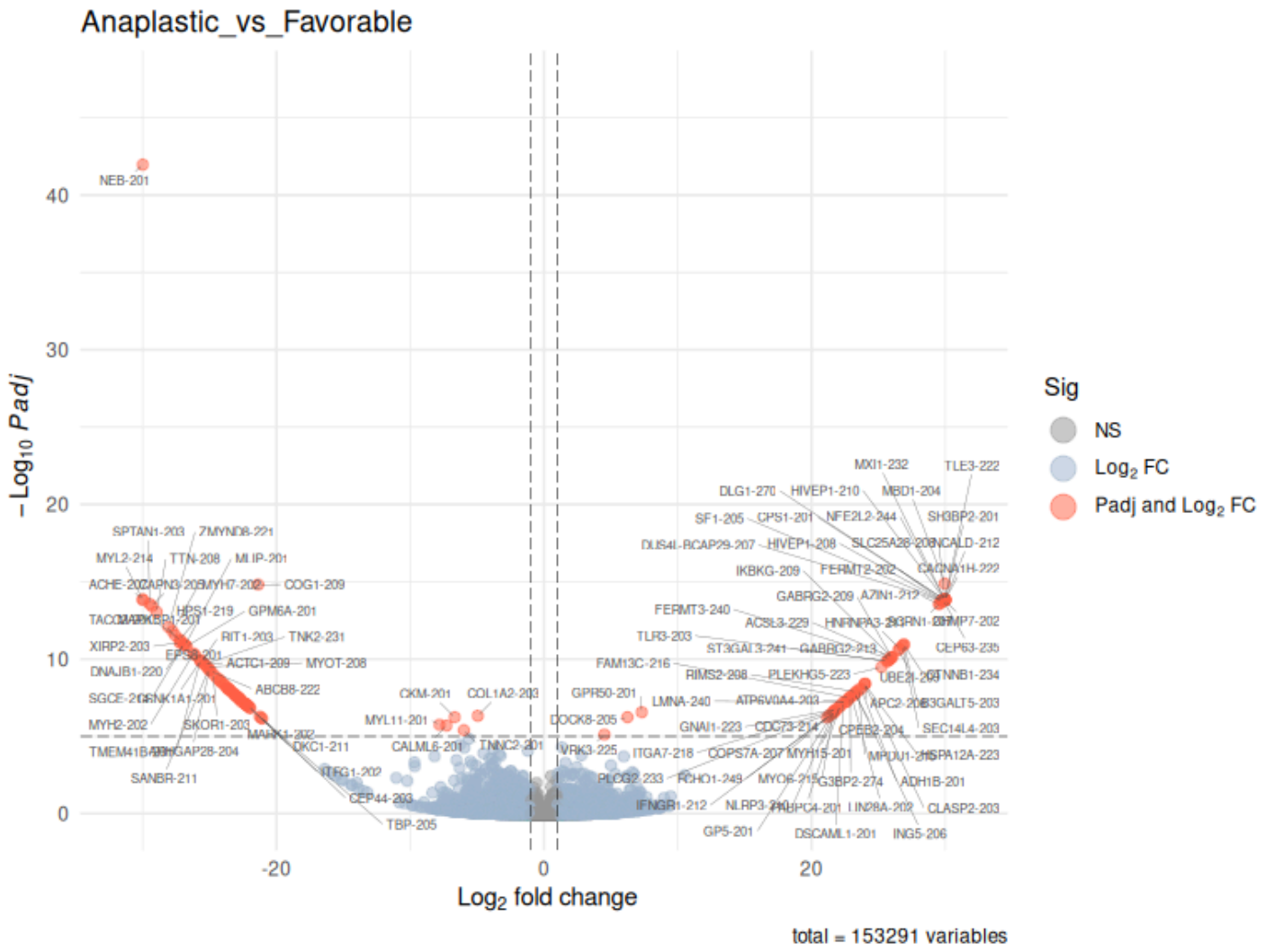
```
openxlsx::write.xlsx(res.transcript.df, file="DESeq2_res.transcript.xlsx")
```

volcano plots

EnhancedVolcano
(https://bioconductor.org/packages/devel/bioc/vignettes/EnhancedVolcano/inst/doc/EnhancedVolcano.html)

Hide

```
p <- EnhancedVolcano(res.transcript.df,
                     lab = res.transcript.df$transcript_name,
                     x = 'log2FoldChange',
                     y = 'padj',
                     ylab = bquote(~-Log[10] ~ italic(Padj)),
                     title = c,
                     subtitle = NULL,
                     cutoffLineCol = "gray10",
                     cutoffLineWidth = 0.2,
                     pointSize = 2,
                     labSize = 2,
                     axisLabSize = 15,
                     titleLabSize = 15,
                     labCol = "gray30",
                     col = c("grey60", "slategray3", "lightpink", "tomato"),
                     legendPosition = 'right',
                     legendLabels=c("NS", expression(Log[2] ~ FC), "Padj", expression(Padj ~ and ~ Log[2] ~
FC)),
                     legendLabSize = 10,
                     max.overlaps = 50,
                     drawConnectors = T,
                     widthConnectors = 0.1,
                     arrowheads = F,
                     colConnectors = "grey30",
                     ) +
    theme_minimal()

save_png_pdf(p, paste0(plotdir, "EnhancedVolcano.transcript.", c), height = 6, width = 8)
```



Kallisto

load kallisto counts over transcripts

Hide

```
kallisto_res.transcript <- read.table("../kallisto/all.est_count.txt.gz", header = T, sep = "\t", row.name
s=1, check.names = F)[-1] # omit column "length"
```

use tximport to aggregate to gene level

Prepare data

read the gtf used for the Kallisto index (full anno, not just “annotation” or “primary_assembly.annotation”)

Hide

```
gencode.v48 <- rtracklayer::readGFF("/storage/research/dbmr_rubin_lab/pipeline/ref/anno/hg38/gencode.v48.c
hr_patch_hapl_scaff.annotation.gtf.gz")
```

did we fetch everything?

Hide

```
sum(!rownames(kallisto_res.transcript) %in% gencode.v48$transcript_id)
```

```
[1] 0
```

more sanity checks

Hide

```
rsem_transcript_list <- read.table("../rsem/all.isoforms.expected_count.results", header = T, sep = "\t")
[[1]]
sum(rsem_transcript_list %in% gencode.v48$transcript_id)
```

```
[1] 387954
```

Hide

```
length(rsem_transcript_list)
```

```
[1] 387954
```

make a data.frame called tx2gene with two columns: 1) transcript ID and 2) gene ID. The column names do not matter but this column order must be used.

Hide

```
tx2gene <- gencode.v48[c("transcript_id", "gene_id")]

# remove NA and duplicate transcripts
tx2gene <- na.omit(tx2gene)
tx2gene <- tx2gene[!duplicated(tx2gene$transcript_id),]
```

gene counts from kallisto TSV files

Hide

```
k_files <- file.path("../kallisto", samples, "abundance.tsv.gz")
names(k_files) <- samples
```

tximport to aggregate to gene level

Hide

```
# we don't need ignoreAfterBar
kallisto_res.gene <- tximport::tximport(k_files, type = "kallisto", tx2gene = tx2gene)

# to df
kallisto_res.gene.df <- as.data.frame(kallisto_res.gene[["abundance"]])
```

compare rsem and kallisto

Hide

```
rsem_res <- read.table("../rsem/all.genes.expected_count.results", header = T, sep = "\t", row.names=1, ch
eck.names = F)[c(-1:-27)] # omit metadata cols

rsem_res.transcript <- read.table("../rsem/all.isoforms.expected_count.results", header = T, sep = "\t", r
ow.names=1, check.names = F)[c(-1:-27)] # omit metadata cols
```

sanity check

Hide

```
identical(colnames(rsem_res.transcript), colnames(kallisto_res.transcript))
```

```
[1] TRUE
```

Note: kallisto has more genes/transcripts than rsem (which are all in kallisto)

Hide

```
rsem_vs_kallisto <- list(transcript = cor(rsem_res.transcript, kallisto_res.transcript[rownames(rsem_res.t
ranscript),], method = "spearman"))

rsem_vs_kallisto[["transcript.coding"]] <- cor(rsem_res_coding.transcript[-1:-27], kallisto_res.transcript
[rownames(rsem_res_coding.transcript),], method = "spearman")

rsem_vs_kallisto[["gene"]] <- cor(rsem_res, kallisto_res.gene.df[rownames(rsem_res),], method = "spearma
n")

rsem_vs_kallisto[["gene.coding"]] <- cor(rsem_res[rownames(rsem_res_coding),], kallisto_res.gene.df[rownam
es(rsem_res_coding),], method = "spearman")
```

Plot correlatoions

Hide

```
for (x in names(rsem_vs_kallisto)) {
    p <- ComplexHeatmap::pheatmap(rsem_vs_kallisto[[x]],
        cluster_rows = F,
        cluster_cols = F,
        main = paste0("RSEM vs. Kallisto", "\n", "counts", "\n", x),
        fontsize_row = 8,
        fontsize_col = 8,
        cellheight = 12,
        cellwidth = 12,
        display_numbers = T,
        fontsize_number = 4,
        heatmap_legend_param = list(title = "Spearman"))

    save_png_pdf(p, paste0(plotdir, "rsem_vs_kallisto.", x), height = 10, width = 10)
    #print(p)
}
```

Save session

Hide

```
save.image(file="session.RData")
```

Hide

```
sessioninfo::session_info()
```

```
── Session info ─────────────────────────────────────────────────────────────────
─────────────────────────────────────────────────────────────────────────────────

──────────────────
 setting  value
 version  R version 4.5.1 (2025-06-13)
 os       Ubuntu 24.04.3 LTS
 system   x86_64, linux-gnu
 ui       RStudio
 language (EN)
 collate  en_US.UTF-8
 ctype    en_US.UTF-8
 tz       Etc/UTC
 date     2026-02-06
 rstudio  2025.09.2+418 Cucumberleaf Sunflower (server)
 pandoc   3.8.2.1 @ /usr/bin/ (via rmarkdown)
 quarto   1.7.32 @ /usr/local/bin/quarto

── Packages ─────────────────────────────────────────────────────────────────────
─────────────────────────────────────────────────────────────────────────────────

──────────────────────
 package              * version  date (UTC) lib source
 abind                  1.4-8    2024-09-12 [1] RSPM (R 4.5.0)
 Biobase              * 2.68.0   2025-04-15 [1] Bioconductor 3.21 (R 4.5.1)
 BiocGenerics         * 0.54.0   2025-04-15 [1] Bioconductor 3.21 (R 4.5.1)
 BiocParallel           1.42.1   2025-06-01 [1] Bioconductor 3.21 (R 4.5.1)
 bslib                  0.9.0    2025-01-30 [2] RSPM (R 4.5.0)
 cachem                 1.1.0    2024-05-16 [2] RSPM (R 4.5.0)
 circlize               0.4.16   2024-02-20 [1] RSPM (R 4.5.0)
 cli                    3.6.5    2025-04-23 [2] RSPM (R 4.5.0)
 clue                   0.3-66   2024-11-13 [1] RSPM (R 4.5.0)
 cluster                2.1.8.1  2025-03-12 [3] CRAN (R 4.5.1)
 codetools              0.2-20   2024-03-31 [3] CRAN (R 4.5.1)
 colorspace             2.1-1    2024-07-26 [1] RSPM (R 4.5.0)
 ComplexHeatmap         2.24.1   2025-06-25 [1] Bioconductor 3.21 (R 4.5.1)
 cowplot                1.2.0    2025-07-07 [1] RSPM (R 4.5.0)
 crayon                 1.5.3    2024-06-20 [2] RSPM (R 4.5.0)
 data.table             1.17.8   2025-07-10 [2] RSPM (R 4.5.0)
 DelayedArray           0.34.1   2025-04-17 [1] Bioconductor 3.21 (R 4.5.1)
 DESeq2               * 1.48.2   2025-08-27 [1] Bioconductor 3.21 (R 4.5.1)
 digest                 0.6.37   2024-08-19 [2] RSPM (R 4.5.0)
 doParallel             1.0.17   2022-02-07 [1] RSPM (R 4.5.0)
 dplyr                  1.1.4    2023-11-17 [2] RSPM (R 4.5.0)
 EnhancedVolcano      * 1.26.0   2025-04-15 [1] Bioconductor 3.21 (R 4.5.1)
 evaluate               1.0.5    2025-08-27 [2] RSPM (R 4.5.0)
 farver                 2.1.2    2024-05-13 [2] RSPM (R 4.5.0)
 fastmap                1.2.0    2024-05-15 [2] RSPM (R 4.5.0)
 fastmatch              1.1-6    2024-12-23 [1] RSPM (R 4.5.0)
 fgsea                * 1.34.2   2025-07-13 [1] Bioconductor 3.21 (R 4.5.1)
 foreach                1.5.2    2022-02-02 [1] RSPM (R 4.5.0)
 fs                     1.6.6    2025-04-12 [2] RSPM (R 4.5.0)
 generics             * 0.1.4    2025-05-09 [2] RSPM (R 4.5.0)
 GenomeInfoDb         * 1.44.1   2025-07-23 [1] Bioconductor 3.21 (R 4.5.1)
```

```
GenomeInfoDbData       1.2.14   2025-08-18 [1] Bioconductor
GenomicRanges        * 1.60.0   2025-04-15 [1] Bioconductor 3.21 (R 4.5.1)
GetoptLong             1.0.5    2020-12-15 [1] RSPM (R 4.5.0)
ggplot2              * 4.0.0    2025-09-11 [2] RSPM (R 4.5.0)
ggplotify            * 0.1.2    2023-08-09 [1] RSPM (R 4.5.0)
ggrepel              * 0.9.6    2024-09-07 [1] RSPM (R 4.5.0)
ggvenn               * 0.1.19   2025-10-08 [1] RSPM
GlobalOptions          0.1.2    2020-06-10 [1] RSPM (R 4.5.0)
glue                   1.8.0    2024-09-30 [2] RSPM (R 4.5.0)
gridGraphics           0.5-1    2020-12-13 [1] RSPM (R 4.5.0)
gtable                 0.3.6    2024-10-25 [2] RSPM (R 4.5.0)
htmltools              0.5.8.1  2024-04-04 [2] RSPM (R 4.5.0)
httr                   1.4.7    2023-08-15 [2] RSPM (R 4.5.0)
IRanges              * 2.42.0   2025-04-15 [1] Bioconductor 3.21 (R 4.5.1)
iterators              1.0.14   2022-02-05 [1] RSPM (R 4.5.0)
jquerylib              0.1.4    2021-04-26 [2] RSPM (R 4.5.0)
jsonlite               2.0.0    2025-03-27 [2] RSPM (R 4.5.0)
knitr                  1.50     2025-03-16 [2] RSPM (R 4.5.0)
labeling               0.4.3    2023-08-29 [2] RSPM (R 4.5.0)
lattice                0.22-7   2025-04-02 [3] CRAN (R 4.5.1)
lifecycle              1.0.4    2023-11-07 [2] RSPM (R 4.5.0)
locfit                 1.5-9.12 2025-03-05 [1] RSPM (R 4.5.0)
magrittr               2.0.4    2025-09-12 [2] RSPM (R 4.5.0)
Matrix                 1.7-3    2025-03-11 [3] CRAN (R 4.5.1)
MatrixGenerics       * 1.20.0   2025-04-15 [1] Bioconductor 3.21 (R 4.5.1)
matrixStats          * 1.5.0    2025-01-07 [1] RSPM (R 4.5.0)
patchwork            * 1.3.2    2025-08-25 [1] RSPM (R 4.5.0)
pheatmap               1.0.13   2025-06-05 [1] RSPM (R 4.5.0)
pillar                 1.11.1   2025-09-17 [2] RSPM (R 4.5.0)
pkgconfig              2.0.3    2019-09-22 [2] RSPM (R 4.5.0)
png                    0.1-8    2022-11-29 [2] RSPM (R 4.5.0)
R6                     2.6.1    2025-02-15 [2] RSPM (R 4.5.0)
rappdirs               0.3.3    2021-01-31 [2] RSPM (R 4.5.0)
RColorBrewer         * 1.1-3    2022-04-03 [2] RSPM (R 4.5.0)
Rcpp                   1.1.0    2025-07-02 [2] RSPM (R 4.5.0)
rjson                  0.2.23   2024-09-16 [1] RSPM (R 4.5.0)
rlang                  1.1.6    2025-04-11 [2] RSPM (R 4.5.0)
rmarkdown              2.30     2025-09-28 [2] RSPM (R 4.5.0)
rsconnect              1.6.0    2025-10-28 [2] RSPM (R 4.5.0)
rstudioapi             0.17.1   2024-10-22 [2] RSPM (R 4.5.0)
S4Arrays               1.8.1    2025-06-01 [1] Bioconductor 3.21 (R 4.5.1)
S4Vectors            * 0.46.0   2025-04-15 [1] Bioconductor 3.21 (R 4.5.1)
S7                     0.2.0    2024-11-07 [1] RSPM (R 4.5.0)
sass                   0.4.10   2025-04-11 [2] RSPM (R 4.5.0)
scales                 1.4.0    2025-04-24 [2] RSPM (R 4.5.0)
sessioninfo            1.2.3    2025-02-05 [2] RSPM (R 4.5.0)
shape                  1.4.6.1  2024-02-23 [1] RSPM (R 4.5.0)
SparseArray            1.8.1    2025-07-23 [1] Bioconductor 3.21 (R 4.5.1)
SummarizedExperiment * 1.38.1   2025-04-30 [1] Bioconductor 3.21 (R 4.5.1)
tibble                 3.3.0    2025-06-08 [2] RSPM (R 4.5.0)
tidyselect             1.2.1    2024-03-11 [2] RSPM (R 4.5.0)
UCSC.utils             1.4.0    2025-04-15 [1] Bioconductor 3.21 (R 4.5.1)
```

```
 vctrs                  0.6.5    2023-12-01 [2] RSPM (R 4.5.0)
 withr                  3.0.2    2024-10-28 [2] RSPM (R 4.5.0)
 xfun                   0.53     2025-08-19 [2] RSPM (R 4.5.0)
 XVector                0.48.0   2025-04-15 [1] Bioconductor 3.21 (R 4.5.1)
 yaml                   2.3.10   2024-07-26 [2] RSPM (R 4.5.0)
 yulab.utils            0.2.1    2025-08-19 [1] RSPM (R 4.5.0)

 [1] /storage/research/dbmr_rubin_lab/R_lib/Rocker_rstudio_4.5.1
 [2] /usr/local/lib/R/site-library
 [3] /usr/local/lib/R/library
 * ─── Packages attached to the search path.

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```