Alveolar Macrophages after Murine Lung Transplant

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## R Markdown

# **BMS353**

# **Bioinformatics**

**Alveolar Macrophages after Murine Lung Transplant - (mouse)**

This is an R Markdown document. Markdown is a simple formatting syntax.

When you click the **Knit** button a document will be generated that includes both content as well as the output of any embedded R code chunks within the document. You can embed an R code chunk like this:

**INTRODUCTION**

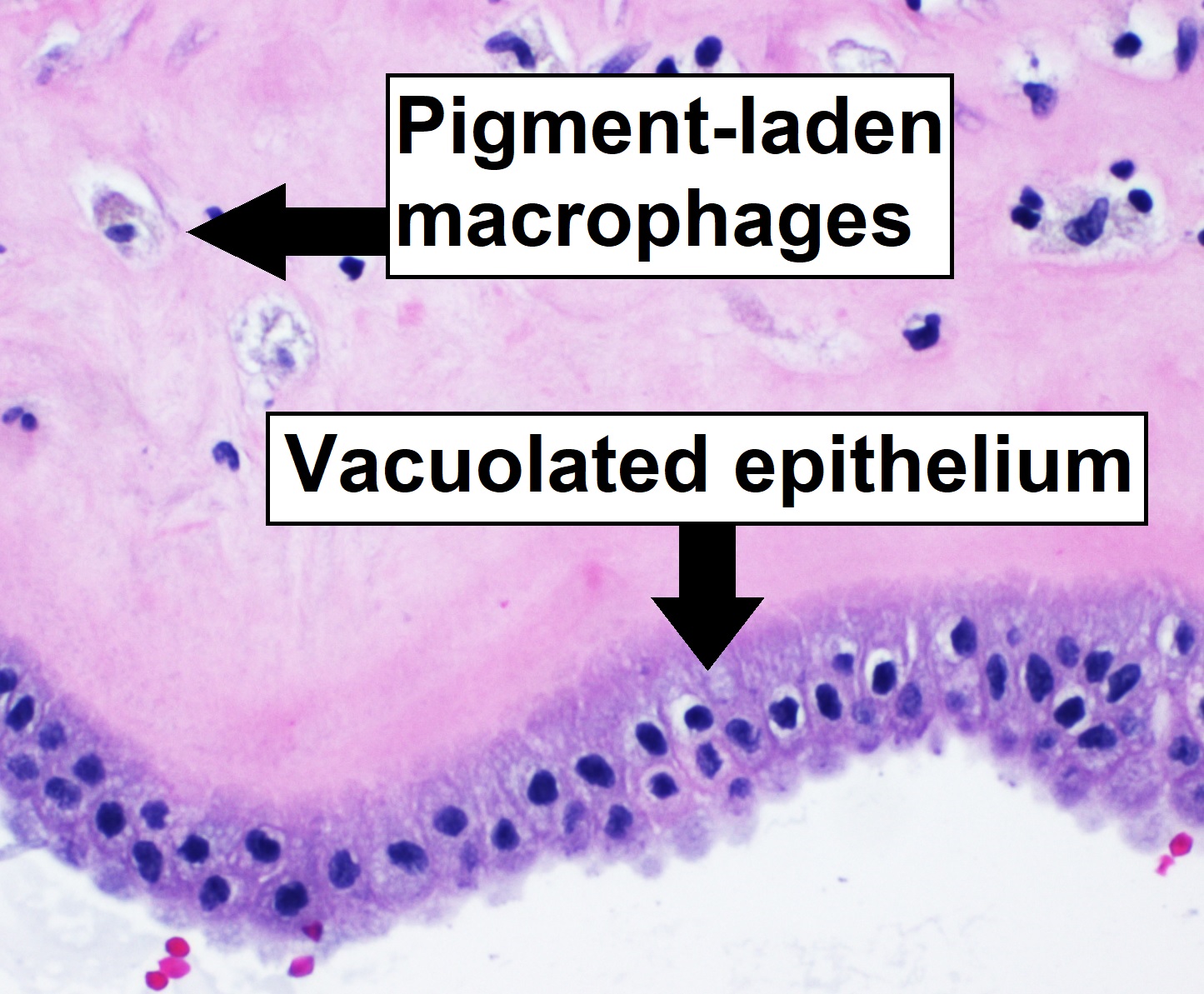


Figure 1 - Image that shows stained macrophage that is found in alveolar sac. Image is just informative but not necessarly relevant to this project

In this report we will analyze data for a research study that covers the following topic: Alveolar Macrophages after Murine Lung Transplant. For this we have the following resources provided:

*METHODS*

`````````````````````````````````````insert text from word

|  |  |
| --- | --- |
| Sample number | Condition |
| 4 | naive |
| 4 | 2h post-perfusion |
| 4 | 24 h post-perfusion |

[GSE116583](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE116583)

[Google drive](https://drive.google.com/drive/folders/1AqYi0Ps5t5xo6XYWXjOtO6eDOMpT1ADE) - Google drive folder with the following content:

* meta\_data
* salmon-quant.sf files
* tx2gene.csv

The project name BMS353-Alveolar-Macrophages—RNA-seq-dataset with all files and the code are available on github in a repository:

[Github](https://github.com/IoanaAndra/BMS353-Alveolar-Macrophages---RNA-seq-dataset)

In order to use the necessary functions and analyze the data, we use some packages available in R:

* readr
* ggplot2
* dplyr

Every chunk of code can be name like this:

Installing all packages necessary for this project in R.

#SECTION FOR PACKAGES  
#installing all necessary packages  
# IF using same R version (4.0.3) and have all packages installed already, all lines of this section can remain commented   
   
# install.packages("readr")  
# install.packages("ggplot2") #\*

\*All code is visible in Rmd file.

*ANALYSIS*

\*\*1. Import raw data (.csv and quant.sf files)\*

CSV and TSV file formats can be stored in variables and display first row of them using head()

With head, we display first rows of data tx2gene.csv and a quant.sf

tx2gene.csv is the transcripts and genes relationship table file

library(readr)  
  
dataPath <- "tx2gene.csv" #asigning value which represent path of file  
  
file.exists(dataPath) #file.exists() return TRUE id file can be found or FALSE if it is not found

## [1] TRUE

tx2GeneFile <- read.csv(file = 'tx2gene.csv') #file with delimiter ","  
quantFile <- read\_tsv(file = 'salmon\_quant/SRR7457551/quant.sf') #file with delimiter "\t"  
head(tx2GeneFile)

## TXNAME GENEID  
## 1 ENSMUST00000193812 ENSMUSG00000102693  
## 2 ENSMUST00000082908 ENSMUSG00000064842  
## 3 ENSMUST00000192857 ENSMUSG00000102851  
## 4 ENSMUST00000161581 ENSMUSG00000089699  
## 5 ENSMUST00000192183 ENSMUSG00000103147  
## 6 ENSMUST00000193244 ENSMUSG00000102348

head(quantFile)

## # A tibble: 6 x 5  
## Name Length EffectiveLength TPM NumReads  
## <chr> <dbl> <dbl> <dbl> <dbl>  
## 1 ENSMUST00000193812 1070 756. 0 0  
## 2 ENSMUST00000082908 110 4 0 0  
## 3 ENSMUST00000162897 4153 3719. 0 0  
## 4 ENSMUST00000159265 2989 2604. 0.0174 2  
## 5 ENSMUST00000070533 3634 3376. 0 0  
## 6 ENSMUST00000192857 480 230 0 0

#view(tx2GeneFile)

Name represents in quant. sf the TXNAME found in tx2gene.csv. But tx2gene.csv contains all 12 samples (in order …51-…62)

Sample txt. presents the full experimental design that was followed. 12 columns for all 12 samples that are found in a salmon output format file that can be further read and accessed using read\_tsv

library(tximport)  
library(readr)  
library(dplyr)  
# Read the sample information into R  
sampleinfo <- read.delim("meta\_data/sampleInfo.txt") #reads in delimited text file  
View(sampleinfo) # view sampleinfo.txt in a new tab  
#glimpse(sampleinfo)  
sampleinfo

## geo\_accession name condition time run  
## 1 GSM3243460 N01\_AM\_Naive naive 0hr SRR7457557  
## 2 GSM3243461 N02\_AM\_Naive naive 0hr SRR7457558  
## 3 GSM3243462 N03\_AM\_Naive naive 0hr SRR7457559  
## 4 GSM3243463 N04\_AM\_Naive naive 0hr SRR7457560  
## 5 GSM3243464 R01\_AM\_Allo2h post\_reperfusion 2hr SRR7457553  
## 6 GSM3243465 R02\_AM\_Allo2h post\_reperfusion 2hr SRR7457554  
## 7 GSM3243466 R03\_AM\_Allo2h post\_reperfusion 2hr SRR7457555  
## 8 GSM3243467 R04\_AM\_Allo2h post\_reperfusion 2hr SRR7457556  
## 9 GSM3243468 R05\_AM\_Allo24h post\_reperfusion 24hr SRR7457551  
## 10 GSM3243469 R06\_AM\_Allo24h post\_reperfusion 24hr SRR7457552  
## 11 GSM3243470 R07\_AM\_Allo24h post\_reperfusion 24hr SRR7457561  
## 12 GSM3243471 R08\_AM\_Allo24h post\_reperfusion 24hr SRR7457562

rownames(sampleinfo) <- sampleinfo$run   
  
dirs <- list.files("salmon\_quant/")  
quant\_files <- list.files("salmon\_quant/",pattern="quant.sf",recursive = TRUE,full.names = TRUE)  
#names(quant\_files) <- dirs  
quant\_files

## [1] "salmon\_quant//SRR7457551/quant.sf" "salmon\_quant//SRR7457552/quant.sf"  
## [3] "salmon\_quant//SRR7457553/quant.sf" "salmon\_quant//SRR7457554/quant.sf"  
## [5] "salmon\_quant//SRR7457555/quant.sf" "salmon\_quant//SRR7457556/quant.sf"  
## [7] "salmon\_quant//SRR7457557/quant.sf" "salmon\_quant//SRR7457558/quant.sf"  
## [9] "salmon\_quant//SRR7457559/quant.sf" "salmon\_quant//SRR7457560/quant.sf"  
## [11] "salmon\_quant//SRR7457561/quant.sf" "salmon\_quant//SRR7457562/quant.sf"

# tx2gene links transcript IDs to gene IDs for summarization  
tx2geneVariable <- read.csv("tx2gene.csv")  
  
txi <- tximport(files=quant\_files, type="salmon", tx2gene=tx2geneVariable)  
  
View(txi) #view txi in a new tab  
  
#inspect salmon output (quant.sf files)  
quants <- read\_tsv(quant\_files[1])  
head(quants)

## # A tibble: 6 x 5  
## Name Length EffectiveLength TPM NumReads  
## <chr> <dbl> <dbl> <dbl> <dbl>  
## 1 ENSMUST00000193812 1070 756. 0 0  
## 2 ENSMUST00000082908 110 4 0 0  
## 3 ENSMUST00000162897 4153 3719. 0 0  
## 4 ENSMUST00000159265 2989 2604. 0.0174 2  
## 5 ENSMUST00000070533 3634 3376. 0 0  
## 6 ENSMUST00000192857 480 230 0 0

# spec(quants)  
   
 filter(quants, quants$TPM ==0) #filter data where TPM = 0

## # A tibble: 60,226 x 5  
## Name Length EffectiveLength TPM NumReads  
## <chr> <dbl> <dbl> <dbl> <dbl>  
## 1 ENSMUST00000193812 1070 756. 0 0  
## 2 ENSMUST00000082908 110 4 0 0  
## 3 ENSMUST00000162897 4153 3719. 0 0  
## 4 ENSMUST00000070533 3634 3376. 0 0  
## 5 ENSMUST00000192857 480 230 0 0  
## 6 ENSMUST00000195335 2819 250 0 0  
## 7 ENSMUST00000192336 2233 250 0 0  
## 8 ENSMUST00000194099 2309 250 0 0  
## 9 ENSMUST00000161581 250 20 0 0  
## 10 ENSMUST00000192973 2057 250 0 0  
## # ... with 60,216 more rows

#print.data.frame(quants) #print quants, maximum output to print in console 200 lines  
   
tail(quants, n = 3)

## # A tibble: 3 x 5  
## Name Length EffectiveLength TPM NumReads  
## <chr> <dbl> <dbl> <dbl> <dbl>  
## 1 ENSMUST00000179077 887 762. 17.0 574.   
## 2 ENSMUST00000189352 548 295. 1.45 18.9  
## 3 ENSMUST00000178569 1083 250 0 0

max(quants$TPM)

## [1] 63095.94

After asigning to quants variable the files

VEZI DACA ACEASTA BUCATA DE PLOT VINE DUPA PCA CU vsd

rpk <- quants$NumReads / quants$EffectiveLength  
scale\_factor <- sum(rpk) / 1e6  
tpm <- rpk / scale\_factor  
  
#define transcript mapping  
  
gtf\_file <- "Mus\_musculus.GRCm38.91.chr.gtf.gz"  
file.exists(gtf\_file)

## [1] TRUE

download.file("ftp://ftp.ensembl.org/pub/release-91/gtf/mus\_musculus/Mus\_musculus.GRCm38.91.chr.gtf.gz",destfile = gtf\_file) #gtf based on organism of interest  
  
# create a database of transcripts  
# Could take a few minutes to run the makeTxDbFromGFF command  
library(GenomicFeatures)  
txdb <- makeTxDbFromGFF(gtf\_file)  
  
  
#specify number of keys and columns  
  
keytypes(txdb)

## [1] "CDSID" "CDSNAME" "EXONID" "EXONNAME" "GENEID" "TXID" "TXNAME"

columns(txdb)

## [1] "CDSCHROM" "CDSEND" "CDSID" "CDSNAME" "CDSPHASE"   
## [6] "CDSSTART" "CDSSTRAND" "EXONCHROM" "EXONEND" "EXONID"   
## [11] "EXONNAME" "EXONRANK" "EXONSTART" "EXONSTRAND" "GENEID"   
## [16] "TXCHROM" "TXEND" "TXID" "TXNAME" "TXSTART"   
## [21] "TXSTRAND" "TXTYPE"

#get names for all transcripts - using keys function  
#compose query - using select function - this will return data frame  
  
k <- keys(txdb, keytype="TXNAME")  
tx\_map <- select(txdb, keys = k, columns="GENEID", keytype = "TXNAME")  
  
#visualise first rows of the transcript map  
head(tx\_map)

## TXNAME GENEID  
## 1 ENSMUST00000193812 ENSMUSG00000102693  
## 2 ENSMUST00000082908 ENSMUSG00000064842  
## 3 ENSMUST00000192857 ENSMUSG00000102851  
## 4 ENSMUST00000161581 ENSMUSG00000089699  
## 5 ENSMUST00000192183 ENSMUSG00000103147  
## 6 ENSMUST00000193244 ENSMUSG00000102348

#use tximport package to import raw data for each of the 12 samples  
  
library(tximport)  
tx2gene <- tx\_map  
# write.csv(tx2gene,file="tx2gene.csv",row.names = FALSE,quote=FALSE)  
txi <- tximport(quant\_files,type="salmon",tx2gene = tx2gene)  
  
table(tx\_map$TXNAME %in% quants$Name)  
  
tx2gene <- tx\_map  
txi <- tximport(quant\_files,type="salmon",tx2gene = tx2gene,ignoreTxVersion = TRUE)  
  
names(txi)  
  
head(txi$counts)   
  
all(rownames(sampleinfo) == colnames(txi$counts))  
  
library(tidyr)  
library(dplyr)  
  
quants <- separate(quants, Name, c("TXNAME","Number"),remove = FALSE)  
head(quants)  
  
  
quants <- left\_join(quants, tx\_map, by="TXNAME")  
head(quants)  
  
tx2gene <- dplyr:::select(quants, Name, GENEID)  
head(tx2gene)  
  
any(is.na(tx2gene$GENEID))  
  
tx2gene <- filter(tx2gene, !is.na(GENEID))  
  
library(tximport)  
txi <- tximport(quant\_files,type="salmon",tx2gene = tx2gene)

Summarising

## # A tibble: 6 x 5  
## Name Length EffectiveLength TPM NumReads  
## <chr> <dbl> <dbl> <dbl> <dbl>  
## 1 ENSMUST00000193812 1070 756. 0 0  
## 2 ENSMUST00000082908 110 4 0 0  
## 3 ENSMUST00000162897 4153 3719. 0 0  
## 4 ENSMUST00000159265 2989 2604. 0.0174 2  
## 5 ENSMUST00000070533 3634 3376. 0 0  
## 6 ENSMUST00000192857 480 230 0 0

## # A tibble: 1 x 4  
## `min(quants$Length)` `max(quants$Length)` `min(quants$Effec~ `max(quants$Effe~  
## <dbl> <dbl> <dbl> <dbl>  
## 1 9 123179 2 119488.

## "1" "Name"  
## "2" "Length"  
## "3" "EffectiveLength"  
## "4" "TPM"  
## "5" "NumReads"

*2. Quality assesment*

Next, testing quality assesment for the raw reads of gene transcripts that were imported with tximport.

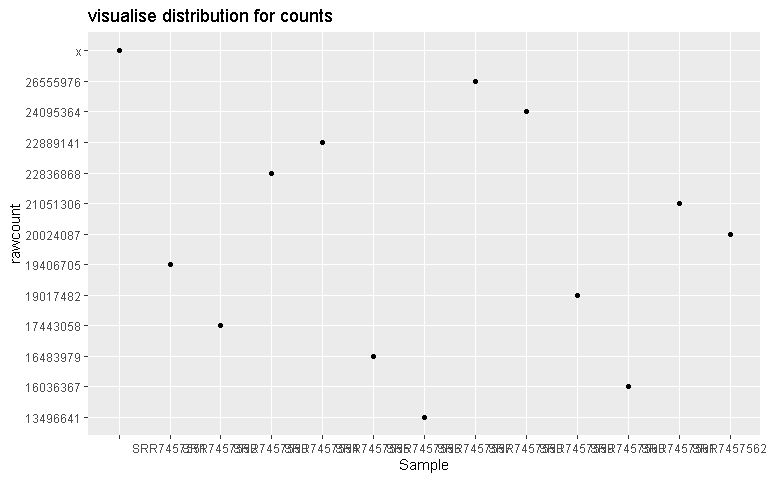
## DataFrame with 12 rows and 5 columns  
## geo\_accession name condition time  
## <character> <character> <factor> <character>  
## SRR7457557 GSM3243460 N01\_AM\_Naive naive 0hr  
## SRR7457558 GSM3243461 N02\_AM\_Naive naive 0hr  
## SRR7457559 GSM3243462 N03\_AM\_Naive naive 0hr  
## SRR7457560 GSM3243463 N04\_AM\_Naive naive 0hr  
## SRR7457553 GSM3243464 R01\_AM\_Allo2h post\_reperfusion 2hr  
## ... ... ... ... ...  
## SRR7457556 GSM3243467 R04\_AM\_Allo2h post\_reperfusion 2hr  
## SRR7457551 GSM3243468 R05\_AM\_Allo24h post\_reperfusion 24hr  
## SRR7457552 GSM3243469 R06\_AM\_Allo24h post\_reperfusion 24hr  
## SRR7457561 GSM3243470 R07\_AM\_Allo24h post\_reperfusion 24hr  
## SRR7457562 GSM3243471 R08\_AM\_Allo24h post\_reperfusion 24hr  
## run  
## <character>  
## SRR7457557 SRR7457557  
## SRR7457558 SRR7457558  
## SRR7457559 SRR7457559  
## SRR7457560 SRR7457560  
## SRR7457553 SRR7457553  
## ... ...  
## SRR7457556 SRR7457556  
## SRR7457551 SRR7457551  
## SRR7457552 SRR7457552  
## SRR7457561 SRR7457561  
## SRR7457562 SRR7457562

we can count the number of reads for each sample and print in the console. This can be written in a csv file.

## [1] "SRR7457557"  
## [1] 26555976  
##   
## [1] "SRR7457558"  
## [1] 24095364  
##   
## [1] "SRR7457559"  
## [1] 19017482  
##   
## [1] "SRR7457560"  
## [1] 16036367  
##   
## [1] "SRR7457553"  
## [1] 22836868  
##   
## [1] "SRR7457554"  
## [1] 22889141  
##   
## [1] "SRR7457555"  
## [1] 16483979  
##   
## [1] "SRR7457556"  
## [1] 13496641  
##   
## [1] "SRR7457551"  
## [1] 19406705  
##   
## [1] "SRR7457552"  
## [1] 17443058  
##   
## [1] "SRR7457561"  
## [1] 21051306  
##   
## [1] "SRR7457562"  
## [1] 20024087

colSums(assay(dds)) # same as above but different output format

This can be plotted into bar chart, scatterplot or boxplot



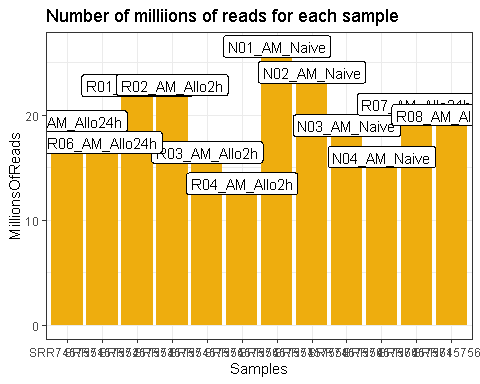


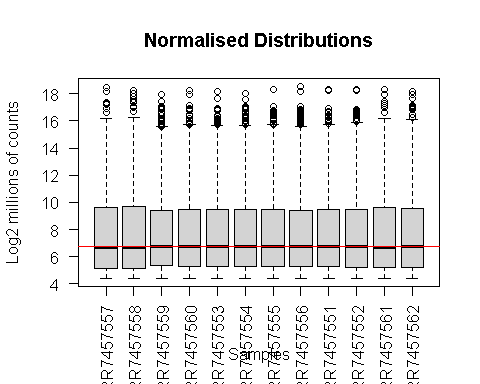
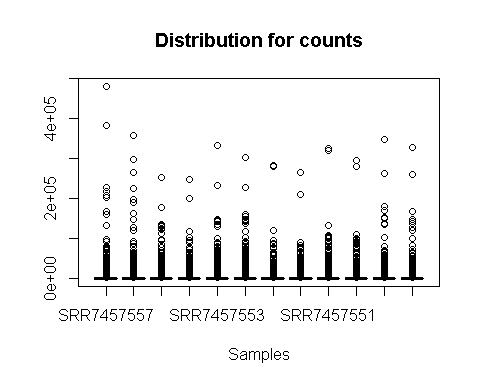
Figure - Bar charts that show number of millions of reads for each of the 12 samples

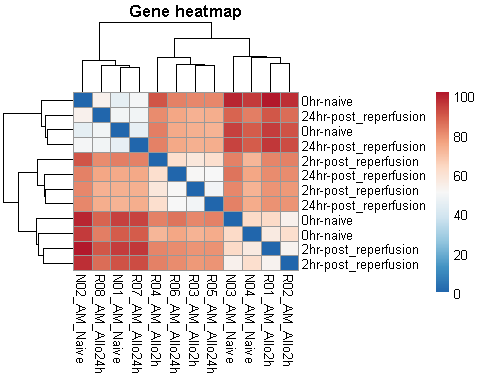
print('Gene from row 3 expressed in this number of samples:')  
sum(is\_expressed[3,])  
  
print('Gene from row 400 expressed in this number of samples:')  
sum(is\_expressed[400,])

keep <- rowSums(assay(dds) >= 5) >= 2  
table(keep)

## keep  
## FALSE TRUE   
## 30585 21748

dds <- dds[keep,] # keep genes

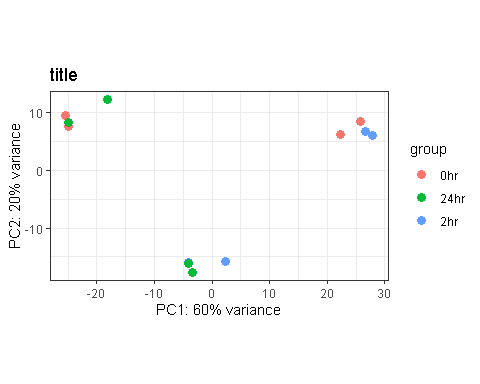




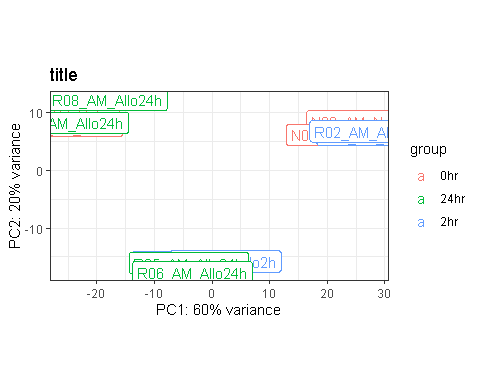
put here caption

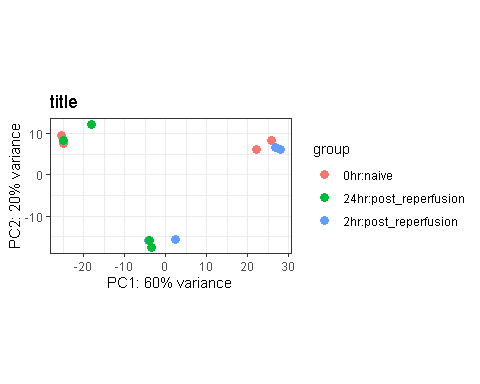
assay(vsd)

## SRR7457557 SRR7457558 SRR7457559 SRR7457560 SRR7457553  
## ENSMUSG00000000001 12.036305 12.106322 11.997854 12.045329 12.041479  
## ENSMUSG00000000028 9.996307 10.262782 7.263084 7.536501 7.251917  
## ENSMUSG00000000037 5.648168 5.517537 4.828132 6.351746 5.188071  
## ENSMUSG00000000056 8.808476 9.337085 6.696121 7.845189 7.761405  
## ENSMUSG00000000058 10.976464 11.253218 10.831330 11.018631 10.924682  
## ENSMUSG00000000078 12.434661 12.399523 12.604306 12.979846 12.986513  
## ENSMUSG00000000085 8.286621 8.130975 8.775195 8.066919 9.043635  
## ENSMUSG00000000088 11.780485 11.849969 11.215712 11.186772 11.198076  
## ENSMUSG00000000093 5.478149 5.381121 5.968794 6.051035 6.202608  
## ENSMUSG00000000094 4.400726 4.812779 5.166022 4.747209 5.354184  
## ENSMUSG00000000120 4.783102 5.045583 4.845244 4.400726 4.400726  
## ENSMUSG00000000127 8.662382 8.778269 7.642229 8.230121 8.123761  
## ENSMUSG00000000131 10.898639 10.842780 10.948162 11.006615 11.232276  
## ENSMUSG00000000134 10.589817 10.450949 10.803828 10.866626 10.591837  
## ENSMUSG00000000142 4.774949 4.803957 5.284886 4.400726 4.671553  
## ENSMUSG00000000148 9.983569 9.978719 9.571171 9.279271 9.100075  
## ENSMUSG00000000149 11.463859 11.548412 11.049271 11.103232 11.261549  
## ENSMUSG00000000154 8.168811 8.271047 8.463078 8.540752 8.485461  
## ENSMUSG00000000167 5.485954 5.924801 5.807333 5.215469 5.413132  
## ENSMUSG00000000168 10.479674 10.522109 10.569232 10.275429 10.419813  
## ENSMUSG00000000171 11.122371 11.302114 10.775315 10.796559 10.810577  
## ENSMUSG00000000184 12.767344 12.489075 13.737329 13.686306 13.439688  
## ENSMUSG00000000194 10.512832 10.259309 10.000633 10.810040 10.603085  
## ENSMUSG00000000204 7.541810 7.963813 8.235711 8.061444 8.312214  
## ENSMUSG00000000223 5.776884 5.329512 5.212841 5.081163 4.940550  
## ENSMUSG00000000244 10.134309 10.149330 8.470426 8.808459 8.731327  
## ENSMUSG00000000247 6.442877 5.781144 5.035679 5.365937 5.203638  
## ENSMUSG00000000253 10.457874 10.631947 10.529651 9.904364 10.204633  
## ENSMUSG00000000266 4.869707 5.358231 4.883207 5.173972 5.079406  
## ENSMUSG00000000275 12.046921 11.764216 12.448633 13.143849 13.538738  
## ENSMUSG00000000276 9.347840 9.105843 9.533584 9.702664 9.930355  
## ENSMUSG00000000278 11.172405 11.298937 10.825117 11.036188 11.023320  
## ENSMUSG00000000282 10.200895 9.907666 9.528521 10.437167 10.609840  
## ENSMUSG00000000290 15.191101 15.239414 15.346417 15.230317 15.385946  
## ENSMUSG00000000295 7.343253 7.317254 7.486747 7.125733 7.374689  
## ENSMUSG00000000296 4.400726 4.785767 5.142680 4.400726 5.040840  
## ENSMUSG00000000301 5.898936 6.260844 5.798556 5.460249 5.650991  
## ENSMUSG00000000303 10.351217 10.221829 10.860427 10.869452 10.655889  
## ENSMUSG00000000305 4.400726 4.400726 4.969347 4.631421 5.575126  
## ENSMUSG00000000308 4.400726 4.400726 5.057457 4.929439 4.400726  
## ENSMUSG00000000318 5.390266 5.617842 5.865651 5.135924 5.375994  
## ENSMUSG00000000320 4.867008 5.209057 5.179435 5.231678 5.043586  
## ENSMUSG00000000325 7.031571 7.015911 7.758859 7.421109 7.134971  
## ENSMUSG00000000326 12.306734 12.454601 12.162063 12.142744 12.031854  
## ENSMUSG00000000339 9.009692 9.100318 8.769548 8.492513 8.587842  
## ENSMUSG00000000340 9.061719 9.258868 9.132424 8.266637 9.153021  
## ENSMUSG00000000346 12.674021 12.650567 12.733489 12.721691 12.911366  
## ENSMUSG00000000355 9.661339 9.758128 9.263598 9.367438 9.243144  
## ENSMUSG00000000359 8.224148 7.800738 8.362612 7.227294 7.145225  
## ENSMUSG00000000365 6.220001 7.592536 7.012473 6.007992 7.238091  
## ENSMUSG00000000374 11.085401 11.369734 10.869898 11.027723 11.281871  
## ENSMUSG00000000378 10.211298 10.151062 9.634891 9.561932 9.667596  
## ENSMUSG00000000384 9.986157 9.865925 10.396520 9.934250 10.044564  
## ENSMUSG00000000386 5.850394 5.666829 5.428182 5.527585 5.745889  
## ENSMUSG00000000392 4.897434 5.371218 5.976800 6.136235 6.060283  
## ENSMUSG00000000399 10.528185 10.767330 10.006947 9.848849 9.909264  
## ENSMUSG00000000409 5.604019 5.261673 6.638314 5.659252 6.022461  
## ENSMUSG00000000411 4.400726 4.400726 4.400726 5.087644 4.812222  
## ENSMUSG00000000420 11.169945 11.405331 10.585868 10.714776 10.901551  
## ENSMUSG00000000439 9.418711 10.111863 9.329411 9.336801 9.733536  
## ENSMUSG00000000440 12.029524 11.916606 11.802423 11.904708 11.325584  
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## ENSMUSG00000036442 9.418604 9.341238  
## [ reached getOption("max.print") -- omitted 13415 rows ]



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 REVEZI AICI DIN TUTORIAL <https://sbc.shef.ac.uk/workshops/2020-02-13-rnaseq-r/rna-seq-preprocessing.nb.html#resources_and_data_files>



put here caption

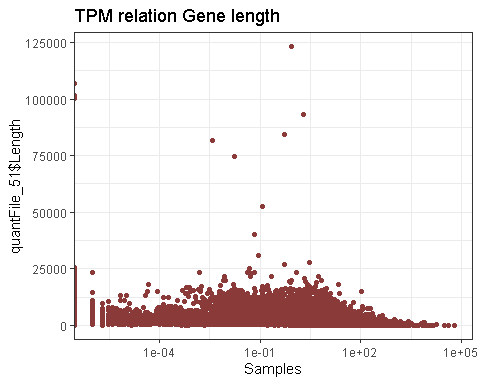
assay(vsd) %>%   
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## 4542 ENSMUSG00000026527 4.769152 5.018798 5.520570 5.157115 5.022753  
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## 7691 11.282340  
## 7692 8.573482  
## [ reached 'max' / getOption("max.print") -- omitted 14056 rows ]

SEE IF YOU DO STATISTICS WITH vsd (above)

*3. Differential Expression*

#   
# sampleinfo\_corrected <- mutate(sampleinfo, time = str\_to\_lower(time)) %>%   
# mutate(condition = str\_trim(condition)) %>%   
# mutate(time = ifelse(Name == "MCL1-DH","basal",time)) %>%   
# mutate(time= ifelse(Name == "MCL1-LA","luminal",time))  
# sampleinfo\_corrected  
# plot\_data <- plotPCA(vsd,intgroup=c("time","condition"),returnData=TRUE)  
# plot\_data <- bind\_cols(plot\_data,sampleinfo\_corrected)



**CONCLUSIONS**

*References*