

# main

July 9, 2021

## 1 Differential Expression with limma-voom pipeline

```
[1]: suppressMessages({library(SummarizedExperiment)
                        library(tidyverse)
                        library(limma)
                        library(edgeR)})
```

### 1.1 Functions

#### 1.1.1 Simple functions

```
[2]: # Function from jaffelab github
merge_rse_metrics <- function(rse) {
  stopifnot(is(rse, 'RangedSummarizedExperiment'))

  rse$overallMapRate = mapply(function(r, n) {
    sum(r*n)/sum(n)
  }, rse$overallMapRate, rse$numReads)
  rse$mitoRate = mapply(function(r, n) {
    sum(r*n)/sum(n)
  }, rse$mitoRate, rse$numMapped)
  rse$rRNA_rate = mapply(function(r, n) {
    sum(r*n)/sum(n)
  }, rse$rRNA_rate, rse$numMapped)
  rse$totalAssignedGene = mapply(function(r, n) {
    sum(r*n)/sum(n)
  }, rse$totalAssignedGene, rse$numMapped)

  rse$numMapped = sapply(rse$numMapped, sum)
  rse$numReads = sapply(rse$numReads, sum)
  rse$numUnmapped = sapply(rse$numUnmapped, sum)
  rse$mitoMapped = sapply(rse$mitoMapped, sum)
  rse$totalMapped = sapply(rse$totalMapped, sum)
  return(rse)
}

save_volcanoPlot <- function(top, label, feature){
```

```

pdf(file=paste0(feature, "/volcanoPlot_", label, ".pdf"), 8, 6)
with(top, plot(logFC, -log10(P.Value), pch=20, cex=0.6))
with(subset(top, adj.P.Val<=0.05), points(logFC, -log10(P.Value),
                                          pch=20, col='red', cex=0.6))
with(subset(top, abs(logFC)>0.50), points(logFC, -log10(P.Value),
                                          pch=20, col='orange', cex=0.6))
with(subset(top, adj.P.Val<=0.05 & abs(logFC)>0.50),
     points(logFC, -log10(P.Value), pch=20, col='green', cex=0.6))
dev.off()
}

save_MApplot <- function(top, label, feature){
  pdf(file=paste0(feature, "/MAplot_", label, ".pdf"), 8, 6)
  with(top, plot(AveExpr, logFC, pch=20, cex=0.5))
  with(subset(top, adj.P.Val<0.05),
       points(AveExpr, logFC, col="red", pch=20, cex=0.5))
  dev.off()
}

extract_de <- function(contrast, label, efit, feature){
  top <- topTable(efit, coef=contrast, number=Inf, sort.by="P")
  top <- top[order(top$P.Value), ]
  top.fdr <- top %>% filter(adj.P.Val<=0.05)
  print(paste("Comparison for:", label))
  print(paste('There are:', dim(top.fdr)[1], 'DE features!'))
  data.table::fwrite(top, file=paste0(feature, "/diffExpr_", label, "_full.
↪txt"),
                    sep='\t', row.names=TRUE)
  data.table::fwrite(top.fdr, file=paste0(feature, "/diffExpr_", label, "_
↪_FDR05.txt"),
                    sep='\t', row.names=TRUE)
  save_volcanoPlot(top, label, feature)
  save_MApplot(top, label, feature)
}

```

### 1.1.2 Cached functions

```

[3]: get_mds <- function(){
  mds_file = "/ceph/projects/v4_phase3_paper/inputs/genotypes/mds/_m/
↪LIBD_Brain_TopMed.mds"
  mds = data.table::fread(mds_file) %>%
    rename_at(.vars = vars(starts_with("C")),
              function(x){sub("C", "snpPC", x)}) %>%
    mutate_if(is.character, as.factor)
  return(mds)
}

```

```

}

memMDS <- memoise::memoise(get_mds)

prep_data <- function(feature){
  sample_file = "/ceph/projects/v4_phase3_paper/inputs/phenotypes/_m/
  ↪merged_phenotypes_full.csv"
  sample_data = data.table::fread(sample_file) %>% select(RNum, New_Dx) %>%
  ↪filter(New_Dx != '')
  counts_lt = list("genes"="/ceph/projects/v4_phase3_paper/inputs/counts/_m/
  ↪caudate_brainseq_phase3_hg38_rseGene_merged_n464.rda",
    "transcripts"="/ceph/projects/v4_phase3_paper/inputs/
  ↪counts/_m/caudate_brainseq_phase3_hg38_rseTx_merged_n464.rda",
    "exons"="/ceph/projects/v4_phase3_paper/inputs/counts/_m/
  ↪caudate_brainseq_phase3_hg38_rseExon_merged_n464.rda",
    "junctions"="/ceph/projects/v4_phase3_paper/inputs/counts/
  ↪_m/caudate_brainseq_phase3_hg38_rseJxn_merged_n464.rda")
  tx_file = "/ceph/projects/v4_phase3_paper/inputs/counts/_m/
  ↪transcripts_counts/caudate_counts.txt"
  load(counts_lt[[feature]])
  if(exists("rse_gene")){
    rse_df = rse_gene
  } else if (exists("rse_tx")){
    rse_df = rse_tx
    counts <- data.table::fread(tx_file) %>%
  ↪column_to_rownames("gencodeTx") %>%
    select(-c(txLength, gencodeID, Symbol, gene_type)) %>% as.matrix
    assays(rse_df)$counts = counts[, colnames(rse_df)]
  } else if (exists("rse_exon")){
    rse_df = rse_exon
  } else {
    rse_df = rse_jxn
  }
  keepIndex = which((rse_df$Dx %in% c("Control", "Schizo")) &
    rse_df$Age > 17 & rse_df$Race %in% c("AA", "CAUC"))
  rse_df = rse_df[, keepIndex]
  rse_df$Dx = factor(rse_df$Dx, levels = c("Control", "Schizo"))
  rse_df$Sex <- factor(rse_df$Sex)
  rse_df <- merge_rse_metrics(rse_df)
  colData(rse_df)$RIN = sapply(colData(rse_df)$RIN, "[", 1)
  rownames(colData(rse_df)) <- sapply(strsplit(rownames(colData(rse_df)),
  ↪"_"), "[", 1)
  pheno = colData(rse_df) %>% as.data.frame %>%
    inner_join(memMDS(), by=c("BrNum"="FID")) %>%
    distinct(RNum, .keep_all = TRUE) %>%
    inner_join(sample_data, by="RNum")

```

```

# Generate DGE list
x <- DGEList(counts=assays(rse_df)$counts[, pheno$RNum],
             genes=rowData(rse_df), samples=pheno)
# Filter by expression
design0 <- model.matrix(~New_Dx, data=x$samples)
keep.x <- filterByExpr(x, design=design0)
x <- x[keep.x, , keep.lib.sizes=FALSE]
print(paste('There are:', sum(keep.x), 'features left!', sep=' '))
# Normalize library size
x <- calcNormFactors(x, method="TMM")
return(x)
}

memo_prepData <- memoise::memoise(prepare_data)

qSV_model <- function(feature){
  x <- memo_prepData(feature)
  # Design matrix
  mod = model.matrix(~New_Dx + Age + Sex + mitoRate + rRNA_rate +
                    totalAssignedGene + RIN + overallMapRate +
                    snpPC1 + snpPC2 + snpPC3, data = x$samples)
  colnames(mod) <- gsub("New_Dx", "", colnames(mod))
  colnames(mod) <- gsub("SexM", "Male", colnames(mod))
  colnames(mod) <- gsub("\\(Intercept\\)", "Intercept", colnames(mod))
  # Load qSV
  qsv_file = "/ceph/projects/v4_phase3_paper/inputs/counts/text_files_counts/
  ↪_m/caudate/qSV_caudate.csv"
  modQsva <- mod %>% as.data.frame %>% rownames_to_column() %>%
    inner_join(data.table::fread(qsv_file), by=c("rowname"="V1")) %>%
    rename_all(list(~str_replace_all(., 'PC', 'qPC')) %>%
    column_to_rownames("rowname") %>% as.matrix
  return(modQsva)
}

memo_qsvModel <- memoise::memoise(qSV_model)

get_voom <- function(feature){
  ### Perform voom
  x <- memo_prepData(feature)
  modQsva <- memo_qsvModel(feature)
  v <- voom(x[, rownames(modQsva)], modQsva, plot=TRUE)
  save(v, file=paste0(feature, '/voomSVA.RData'))
  return(v)
}

memo_voom <- memoise::memoise(get_voom)

```

```

cal_res <- function(feature){
  ### Calculate residuals
  v <- memo_voom(feature)
  null_model <- v$design %>% as.data.frame %>%
    select(-c("Schizo_AP", "Schizo_noAP")) %>% as.matrix
  fit_res <- lmFit(v, design=null_model)
  res = v$E - ( fit_res$coefficients %*% t(null_model) )
  res_sd = apply(res, 1, sd)
  res_mean = apply(res, 1, mean)
  res_norm = (res - res_mean) / res_sd
  write.table(res_norm, file=paste0(feature, '/residualized_expression.tsv'),
    sep="\t", quote=FALSE)
}

memo_res <- memoise::memoise(cal_res)

fit_voom <- function(feature){
  v <- memo_voom(feature)
  modQsva <- memo_qsvModel(feature)
  fit0 <- lmFit(v, modQsva)
  contr.matrix <- makeContrasts(CTLvsSZ_noAP = Schizo_noAP,
                                CTLvsSZ_AP = Schizo_AP,
                                levels=colnames(modQsva))

  fit <- contrasts.fit(fit0, contrasts=contr.matrix)
  esv <- eBayes(fit)
  return(esv)
}

memo_efit <- memoise::memoise(fit_voom)

```

## 1.2 Differential Expression Analysis

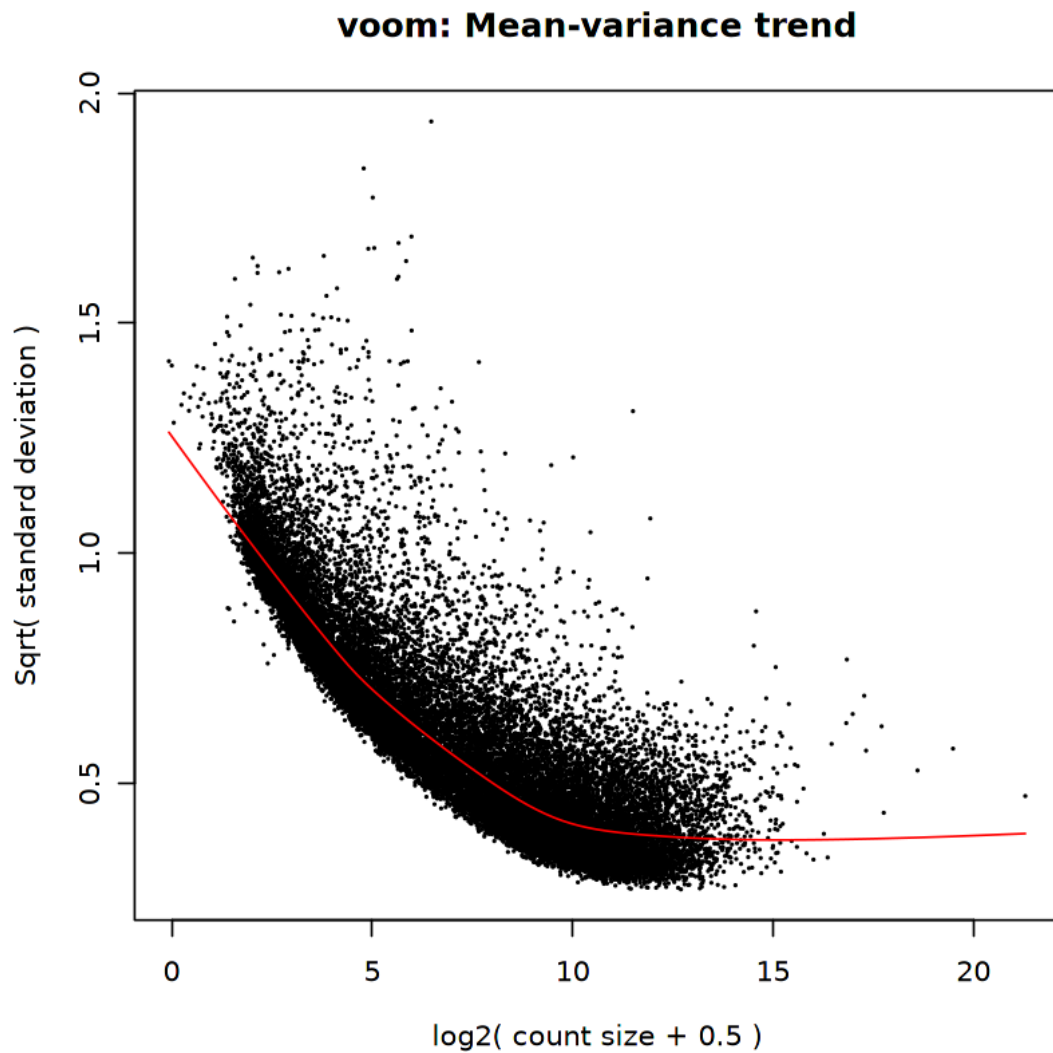
```

[4]: groups = c("sz_noAPVctl", "sz_APVctl")

for(feature in c('genes', 'transcripts', 'junctions', 'exons')){
  dir.create(feature)
  # Preform voom
  v <- memo_voom(feature)
  # Fit model and apply eBayes
  efit = memo_efit(feature)
  # Save differential expression
  for(label in seq_along(groups)){
    extract_de(label, groups[label], efit, feature)
  }
  # Calculate residuals
  memo_res(feature)
}

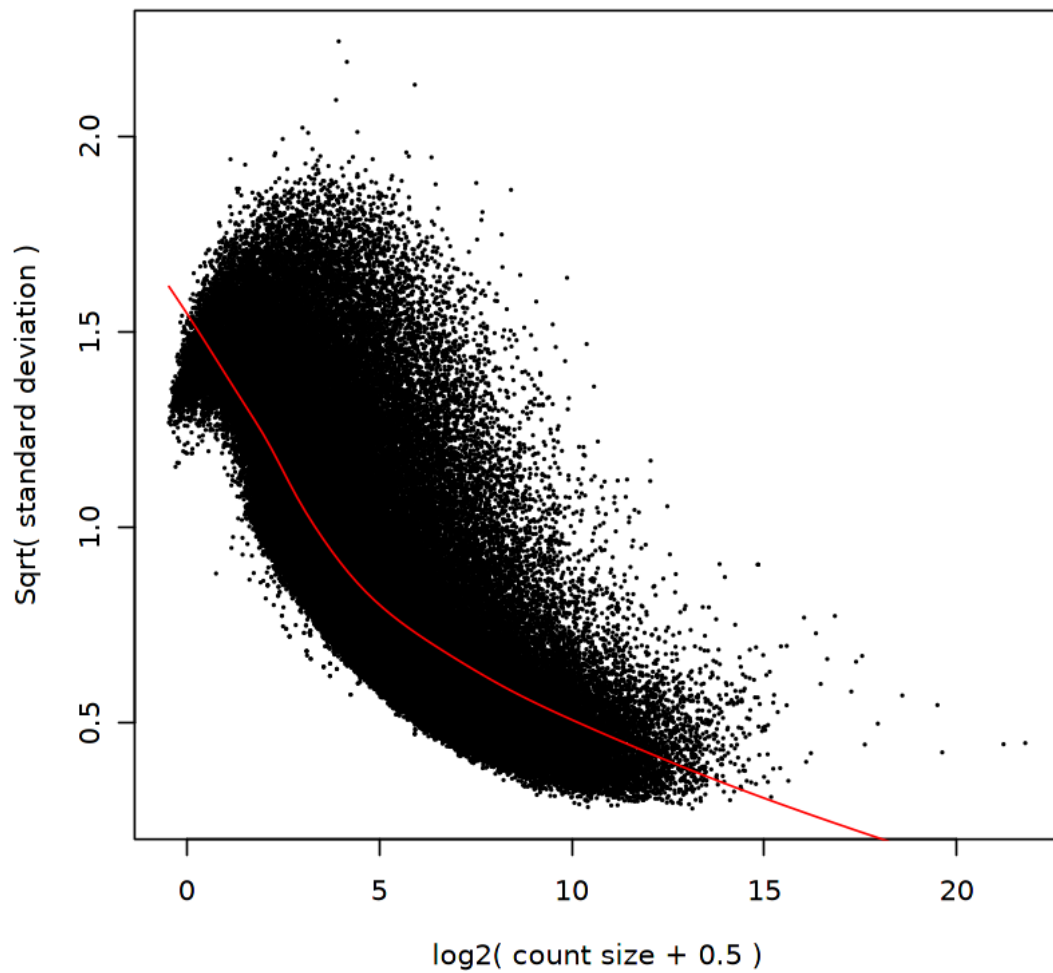
```

```
[1] "There are: 25318 features left!"  
[1] "Comparison for: sz_noAPVctl"  
[1] "There are: 667 DE features!"  
[1] "Comparison for: sz_APVctl"  
[1] "There are: 2692 DE features!"  
[1] "There are: 121961 features left!"  
[1] "Comparison for: sz_noAPVctl"  
[1] "There are: 192 DE features!"  
[1] "Comparison for: sz_APVctl"  
[1] "There are: 1745 DE features!"
```

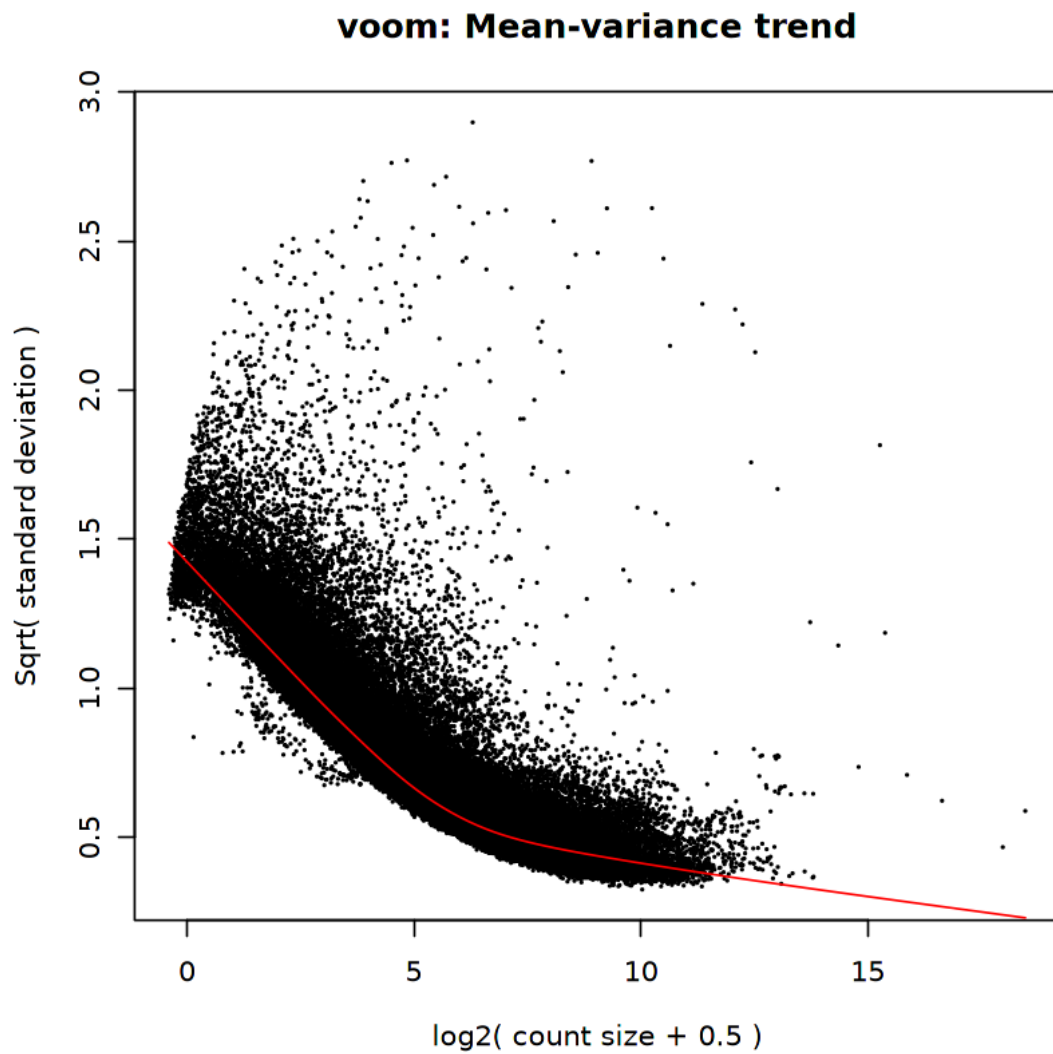


```
[1] "There are: 176134 features left!"
```

### voom: Mean-variance trend

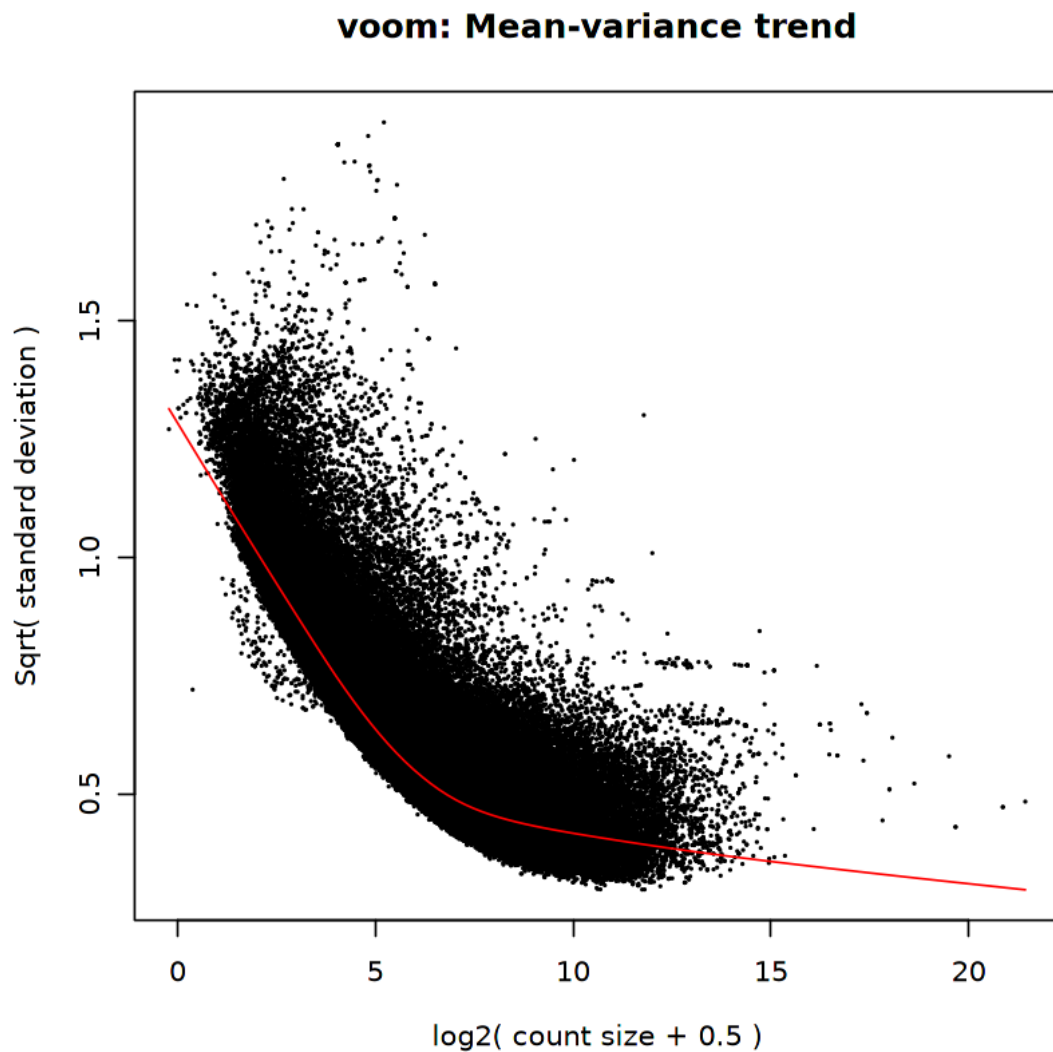


```
[1] "Comparison for: sz_noAPVctl"
[1] "There are: 734 DE features!"
[1] "Comparison for: sz_APVctl"
[1] "There are: 5924 DE features!"
[1] "There are: 384396 features left!"
[1] "There are: 384396 features left!"
```



```
[1] "There are: 384396 features left!"  
[1] "Comparison for: sz_noAPVctl"  
[1] "There are: 3042 DE features!"  
[1] "Comparison for: sz_APVctl"  
[1] "There are: 18809 DE features!"  
[1] "There are: 384396 features left!"
```





### 1.3 Reproducibility Information

```
[5]: Sys.time()
      proc.time()
      options(width = 120)
      sessioninfo::session_info()
```

```
[1] "2021-07-09 15:53:28 EDT"
```

```
      user  system elapsed
4160.652  185.649 3663.952
```

```
Session info
setting  value
```

```

version R version 4.0.3 (2020-10-10)
os      Arch Linux
system  x86_64, linux-gnu
ui      X11
language (EN)
collate en_US.UTF-8
ctype   en_US.UTF-8
tz      America/New_York
date    2021-07-09

```

#### Packages

package	* version	date	lib	source
assertthat	0.2.1	2019-03-21	[1]	CRAN (R 4.0.2)
backports	1.2.1	2020-12-09	[1]	CRAN (R 4.0.2)
base64enc	0.1-3	2015-07-28	[1]	CRAN (R 4.0.2)
Biobase	* 2.50.0	2020-10-27	[1]	Bioconductor
BiocGenerics	* 0.36.1	2021-04-16	[1]	Bioconductor
bitops	1.0-7	2021-04-24	[1]	CRAN (R 4.0.3)
broom	0.7.8	2021-06-24	[1]	CRAN (R 4.0.3)
cachem	1.0.5	2021-05-15	[1]	CRAN (R 4.0.3)
Cairo	1.5-12.2	2020-07-07	[1]	CRAN (R 4.0.2)
cellranger	1.1.0	2016-07-27	[1]	CRAN (R 4.0.2)
cli	3.0.0	2021-06-30	[1]	CRAN (R 4.0.3)
colorspace	2.0-2	2021-06-24	[1]	CRAN (R 4.0.3)
crayon	1.4.1	2021-02-08	[1]	CRAN (R 4.0.3)
data.table	1.14.0	2021-02-21	[1]	CRAN (R 4.0.3)
DBI	1.1.1	2021-01-15	[1]	CRAN (R 4.0.2)
dbplyr	2.1.1	2021-04-06	[1]	CRAN (R 4.0.3)
DelayedArray	0.16.3	2021-03-24	[1]	Bioconductor
digest	0.6.27	2020-10-24	[1]	CRAN (R 4.0.2)
dplyr	* 1.0.7	2021-06-18	[1]	CRAN (R 4.0.3)
edgeR	* 3.32.1	2021-01-14	[1]	Bioconductor
ellipsis	0.3.2	2021-04-29	[1]	CRAN (R 4.0.3)
evaluate	0.14	2019-05-28	[1]	CRAN (R 4.0.2)
fansi	0.5.0	2021-05-25	[1]	CRAN (R 4.0.3)
fastmap	1.1.0	2021-01-25	[1]	CRAN (R 4.0.2)
forcats	* 0.5.1	2021-01-27	[1]	CRAN (R 4.0.2)
fs	1.5.0	2020-07-31	[1]	CRAN (R 4.0.2)
generics	0.1.0	2020-10-31	[1]	CRAN (R 4.0.2)
GenomeInfoDb	* 1.26.7	2021-04-08	[1]	Bioconductor
GenomeInfoDbData	1.2.4	2021-02-02	[1]	Bioconductor
GenomicRanges	* 1.42.0	2020-10-27	[1]	Bioconductor
ggplot2	* 3.3.5	2021-06-25	[1]	CRAN (R 4.0.3)
glue	1.4.2	2020-08-27	[1]	CRAN (R 4.0.2)
gtable	0.3.0	2019-03-25	[1]	CRAN (R 4.0.2)
haven	2.4.1	2021-04-23	[1]	CRAN (R 4.0.3)
hms	1.1.0	2021-05-17	[1]	CRAN (R 4.0.3)
htmltools	0.5.1.1	2021-01-22	[1]	CRAN (R 4.0.2)

httr	1.4.2	2020-07-20	[1]	CRAN (R 4.0.2)
IRanges	* 2.24.1	2020-12-12	[1]	Bioconductor
IRdisplay	1.0	2021-01-20	[1]	CRAN (R 4.0.2)
IRkernel	1.2	2021-05-11	[1]	CRAN (R 4.0.3)
jsonlite	1.7.2	2020-12-09	[1]	CRAN (R 4.0.2)
lattice	0.20-41	2020-04-02	[2]	CRAN (R 4.0.3)
lifecycle	1.0.0	2021-02-15	[1]	CRAN (R 4.0.3)
limma	* 3.46.0	2020-10-27	[1]	Bioconductor
locfit	1.5-9.4	2020-03-25	[1]	CRAN (R 4.0.2)
lubridate	1.7.10	2021-02-26	[1]	CRAN (R 4.0.3)
magrittr	2.0.1	2020-11-17	[1]	CRAN (R 4.0.2)
Matrix	1.3-4	2021-06-01	[1]	CRAN (R 4.0.3)
MatrixGenerics	* 1.2.1	2021-01-30	[1]	Bioconductor
matrixStats	* 0.59.0	2021-06-01	[1]	CRAN (R 4.0.3)
memoise	2.0.0	2021-01-26	[1]	CRAN (R 4.0.2)
modelr	0.1.8	2020-05-19	[1]	CRAN (R 4.0.2)
munsell	0.5.0	2018-06-12	[1]	CRAN (R 4.0.2)
pbdZMQ	0.3-5	2021-02-10	[1]	CRAN (R 4.0.3)
pillar	1.6.1	2021-05-16	[1]	CRAN (R 4.0.3)
pkgconfig	2.0.3	2019-09-22	[1]	CRAN (R 4.0.2)
purrr	* 0.3.4	2020-04-17	[1]	CRAN (R 4.0.2)
R6	2.5.0	2020-10-28	[1]	CRAN (R 4.0.2)
Rcpp	1.0.7	2021-07-07	[1]	CRAN (R 4.0.3)
RCurl	1.98-1.3	2021-03-16	[1]	CRAN (R 4.0.3)
readr	* 1.4.0	2020-10-05	[1]	CRAN (R 4.0.2)
readxl	1.3.1	2019-03-13	[1]	CRAN (R 4.0.2)
repr	1.1.3	2021-01-21	[1]	CRAN (R 4.0.2)
reprex	2.0.0	2021-04-02	[1]	CRAN (R 4.0.3)
rlang	0.4.11	2021-04-30	[1]	CRAN (R 4.0.3)
rstudioapi	0.13	2020-11-12	[1]	CRAN (R 4.0.2)
rvest	1.0.0	2021-03-09	[1]	CRAN (R 4.0.3)
S4Vectors	* 0.28.1	2020-12-09	[1]	Bioconductor
scales	1.1.1	2020-05-11	[1]	CRAN (R 4.0.2)
sessioninfo	1.1.1	2018-11-05	[1]	CRAN (R 4.0.2)
stringi	1.6.2	2021-05-17	[1]	CRAN (R 4.0.3)
stringr	* 1.4.0	2019-02-10	[1]	CRAN (R 4.0.2)
SummarizedExperiment	* 1.20.0	2020-10-27	[1]	Bioconductor
tibble	* 3.1.2	2021-05-16	[1]	CRAN (R 4.0.3)
tidyr	* 1.1.3	2021-03-03	[1]	CRAN (R 4.0.3)
tidyselect	1.1.1	2021-04-30	[1]	CRAN (R 4.0.3)
tidyverse	* 1.3.1	2021-04-15	[1]	CRAN (R 4.0.3)
utf8	1.2.1	2021-03-12	[1]	CRAN (R 4.0.3)
uuid	0.1-4	2020-02-26	[1]	CRAN (R 4.0.2)
vctrs	0.3.8	2021-04-29	[1]	CRAN (R 4.0.3)
withr	2.4.2	2021-04-18	[1]	CRAN (R 4.0.3)
xml2	1.3.2	2020-04-23	[1]	CRAN (R 4.0.2)
XVector	0.30.0	2020-10-27	[1]	Bioconductor
zlibbioc	1.36.0	2020-10-27	[1]	Bioconductor

```
[1] /home/jbenja13/R/x86_64-pc-linux-gnu-library/4.0
[2] /usr/lib/R/library
```