

main

August 12, 2021

1 Differential Expression with limma-voom pipeline - Genes

```
[1]: suppressMessages({library(SummarizedExperiment)
  library(data.table)
  library(tidyverse)
  library(memoise)
  library(limma)
  library(edgeR)
  library(sva)})}
```

1.1 Prepare Data

1.2 Functions

1.2.1 General functions

```
[2]: # Function from jaffelab github
merge_rse_metrics <- function(rse) {
  stopifnot(is(rse, 'RangedSummarizedExperiment'))
  stopifnot(
    c('concordMapRate', 'overallMapRate', 'mitoRate', 'rRNA_rate',
      'totalAssignedGene', 'numMapped', 'numReads', 'numUnmapped',
      'mitoMapped', 'totalMapped') %in%
      colnames(SummarizedExperiment::colData(rse))
  )

  stopifnot(all(sapply(c(
    'concordMapRate', 'overallMapRate', 'mitoRate', 'rRNA_rate',
    'totalAssignedGene', 'numMapped', 'numReads', 'numUnmapped',
    'mitoMapped', 'totalMapped'), function(var) {
      is(colData(rse)[, var], 'List')
    })
  ))

  rse$concordMapRate = mapply(function(r, n) {
    sum(r*n)/sum(n)
  }, rse$concordMapRate, rse$numReads)
  rse$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)
  }
}

```

```

[3]: save_volcanoPlot <- function(top, label, dirname){
  pdf(file=paste0(dirname, "/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, dirname){
  pdf(file=paste0(dirname, "/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, seed_int){
  dirname = paste0("permutation_", stringr::str_pad(seed_int, 2, pad = "0"))
  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!'))
}

```

```

fwrite(top,
       file=paste0(dirname, "/diffExpr_", label, "_full.txt"),
       sep='\t', row.names=TRUE)
fwrite(top.fdr,
       file=paste0(dirname, "/diffExpr_", label, "_FDR05.txt"),
       sep='\t', row.names=TRUE)
save_volcanoPlot(top, label, dirname)
save_MApplot(top, label, dirname)
}

```

1.2.2 Cached functions

```

[4]: load_counts <- function(){
      counts_file = paste0("/ceph/projects/v3_phase3_paper/inputs/phase2/_m/
      ↪count_data/",
                           "hippo_brainseq_phase2_hg38_rseGene_merged_n447.rda")

      load(counts_file)
      rse_df = rse_gene
      return(rse_df)
    }
memCounts <- memoise(load_counts)

get_random_samples <- function(seed_int, new_dir=TRUE){
  set.seed(seed_int + 113) # seed for reproducibility
  dirname = paste0("permutation_", stringr::str_pad(seed_int, 2, pad = "0"))
  if(new_dir){
    dir.create(dirname)
  }
  rse_df <- memCounts()
  keepIndex = which((rse_df$Dx %in% c("Control", "Schizo")) &
                    (rse_df$Age > 17) & (rse_df$Sex == "M") &
                    (rse_df$Race %in% c("AA", "CAUC")))
  snames = sample(keepIndex, 121, replace=FALSE) # subsampling to Female N_
  ↪(sample size)
  return(snames)
}
memSamples <- memoise(get_random_samples)

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(seed_int){
  rse_df <- memCounts()
  keepIndex <- memSamples(seed_int)
  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)
  rse_df$ERCCsumLogErr <- mapply(function(r, n) {
    sum(r * n)/sum(n)
  }, rse_df$ERCCsumLogErr, rse_df$numReads)
  colData(rse_df)$RIN = sapply(colData(rse_df)$RIN, "[", 1)
  rownames(colData(rse_df)) <- sapply(strsplit(rownames(colData(rse_df)),
↪ "_"), "[", 1)
  pheno = colData(rse_df) %>% as.data.frame %>%
    inner_join(memMDS(), by=c("BrNum"="FID")) %>%
    distinct(RNum, .keep_all = TRUE)
  # Generate DGE list
  x <- DGEList(counts=assays(rse_df)$counts[, pheno$RNum],
    genes=rowData(rse_df), samples=pheno)
  # Filter by expression
  design0 <- model.matrix(~Dx, data=x$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(prepare_data)

SVA_model <- function(seed_int){
  x <- memo_prepData(seed_int)
  # Design matrix
  mod = model.matrix(~Dx + Age + mitoRate + rRNA_rate + RIN +
    totalAssignedGene + overallMapRate + ERCCsumLogErr +
    snpPC1 + snpPC2 + snpPC3, data=x$samples)
  colnames(mod) <- gsub("Dx", "", colnames(mod))
  colnames(mod) <- gsub("\\(Intercept\\)", "Intercept", colnames(mod))
  # Calculated SVs
  null.model = mod %>% as.data.frame %>% select(-c("Schizo")) %>% as.matrix
  n.sv <- num.sv(x$counts, mod, method="be")
  svobj <- svaseq(x$counts, mod, null.model, n.sv=n.sv)
  if(svobj$sv == 0){
    modQsva <- mod
  } else {

```

```

    modQsva <- cbind(mod, svobj$sv)
    len.d <- length(colnames(modQsva))
    colnames(modQsva)[((len.d - n.sv)+1):len.d] <- make.names(paste0("sv", 1:
    ↪n.sv))
  }
  return(modQsva)
}
memo_svaModel <- memoise(SVA_model)

get_voom <- function(seed_int){
  ### Perform voom
  x <- memo_prepData(seed_int)
  modQsva <- memo_svaModel(seed_int)
  v <- voom(x[, rownames(modQsva)], modQsva, plot=TRUE)
  return(v)
}
memo_voom <- memoise(get_voom)

cal_res <- function(seed_int){
  ### Calculate residuals
  v <- memo_voom(seed_int)
  null_model <- v$design %>% as.data.frame %>% select(-c("Schizo")) %>% as.
  ↪matrix
  fit_res <- lmFit(v, design=null_model)
  res = v$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
  dirname = paste0("permutation_", stringr::str_pad(seed_int, 2, pad = "0"))
  write.table(res_norm, file=paste0(dirname, '/residualized_expression.tsv'),
    sep="\t", quote=FALSE)
}
memo_res <- memoise(cal_res)

fit_voom <- function(seed_int){
  v <- memo_voom(seed_int)
  modQsva <- memo_svaModel(seed_int)
  fit0 <- lmFit(v, modQsva)
  contr.matrix <- makeContrasts(CtrlvsSZ = Schizo,
    levels=colnames(modQsva))
  fit <- contrasts.fit(fit0, contrasts=contr.matrix)
  esv <- eBayes(fit)
  return(esv)
}
memo_efit <- memoise(fit_voom)

```

1.3 Differential Expression Analysis

```
[5]: for(seed_int in seq(1, 10)){  
      # Preform voom  
      v <- memo_voom(seed_int)  
      dirname = paste0("permutation_", stringr::str_pad(seed_int, 2, pad = "0"))  
      save(v, file=paste0(dirname, '/voomSVA.RData'))  
      # Fit model and apply eBayes  
      efit = memo_efit(seed_int)  
      # Save differential expression  
      extract_de(1, "CtrlvsSZ", efit, seed_int)  
      # Calculate residuals  
      memo_res(seed_int)  
}
```

```
[1] "There are: 22488 features left!"
```

```
Number of significant surrogate variables is: 1
```

```
Iteration (out of 5 ):1 2 3 4 5
```

```
Warning message in if (svobj$sv == 0) {:
```

```
"the condition has length > 1 and only the first element will be used"
```

```
[1] "Comparison for: CtrlvsSZ"
```

```
[1] "There are: 1 DE features!"
```

```
[1] "There are: 22615 features left!"
```

```
Number of significant surrogate variables is: 1
```

```
Iteration (out of 5 ):1 2 3 4 5
```

```
Warning message in if (svobj$sv == 0) {:
```

```
"the condition has length > 1 and only the first element will be used"
```

```
[1] "Comparison for: CtrlvsSZ"
```

```
[1] "There are: 12 DE features!"
```

```
[1] "There are: 22901 features left!"
```

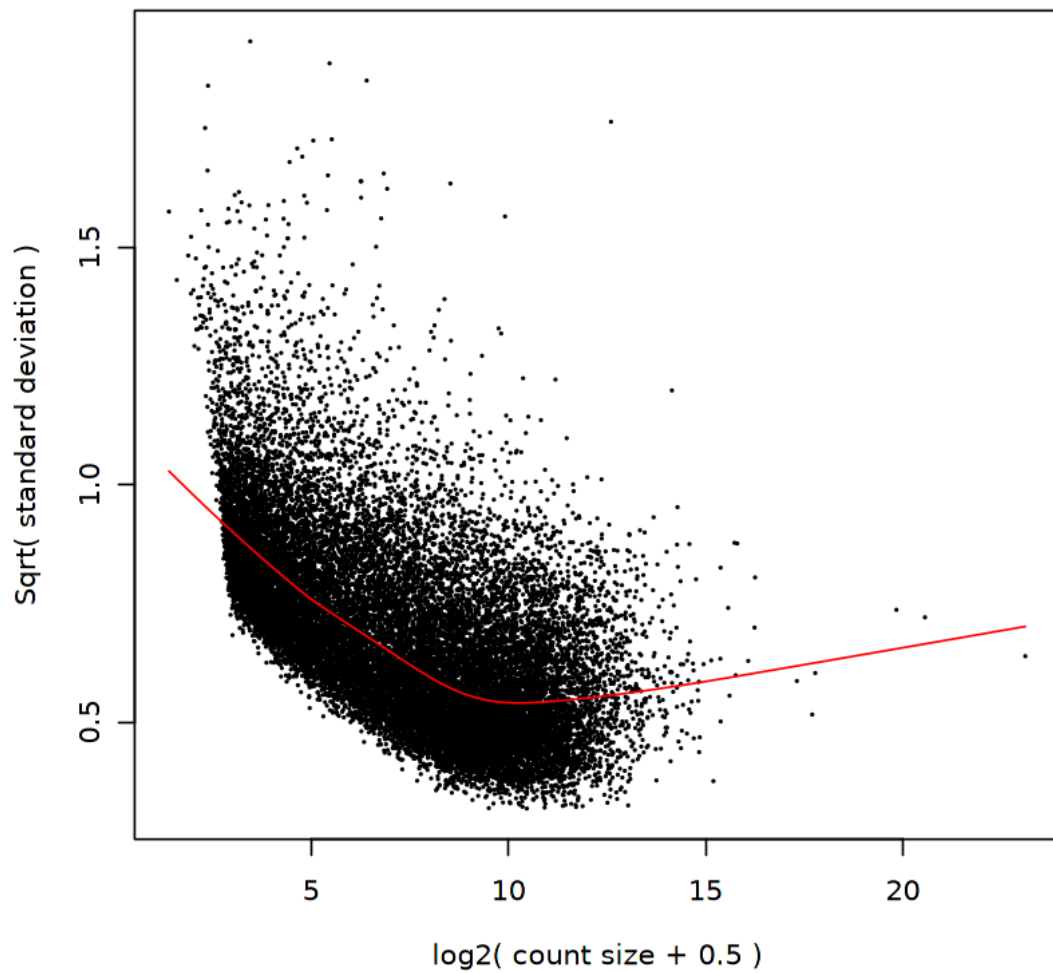
```
Number of significant surrogate variables is: 1
```

```
Iteration (out of 5 ):1 2 3 4 5
```

```
Warning message in if (svobj$sv == 0) {:
```

```
"the condition has length > 1 and only the first element will be used"
```

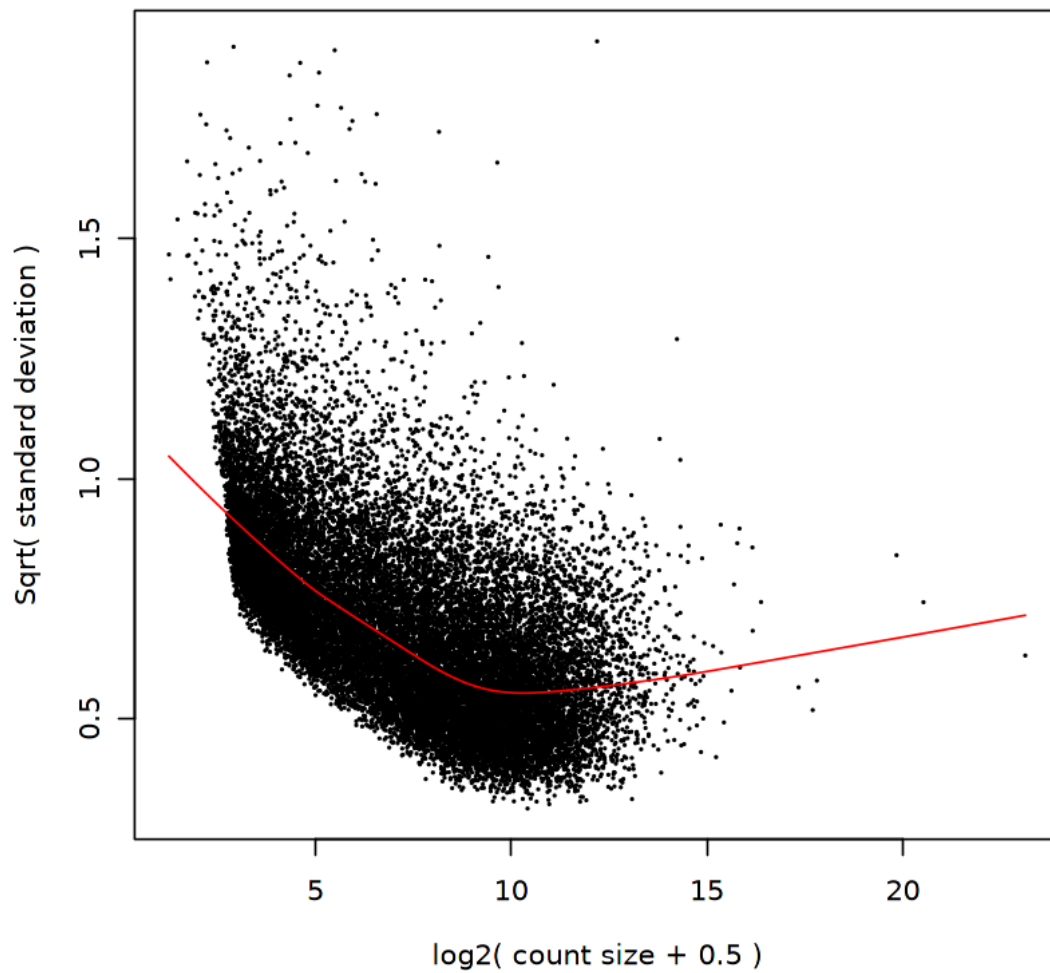
voom: Mean-variance trend



```
[1] "Comparison for: CtrlvsSZ"
[1] "There are: 0 DE features!"
[1] "There are: 22579 features left!"
Number of significant surrogate variables is: 1
Iteration (out of 5 ):1 2 3 4 5

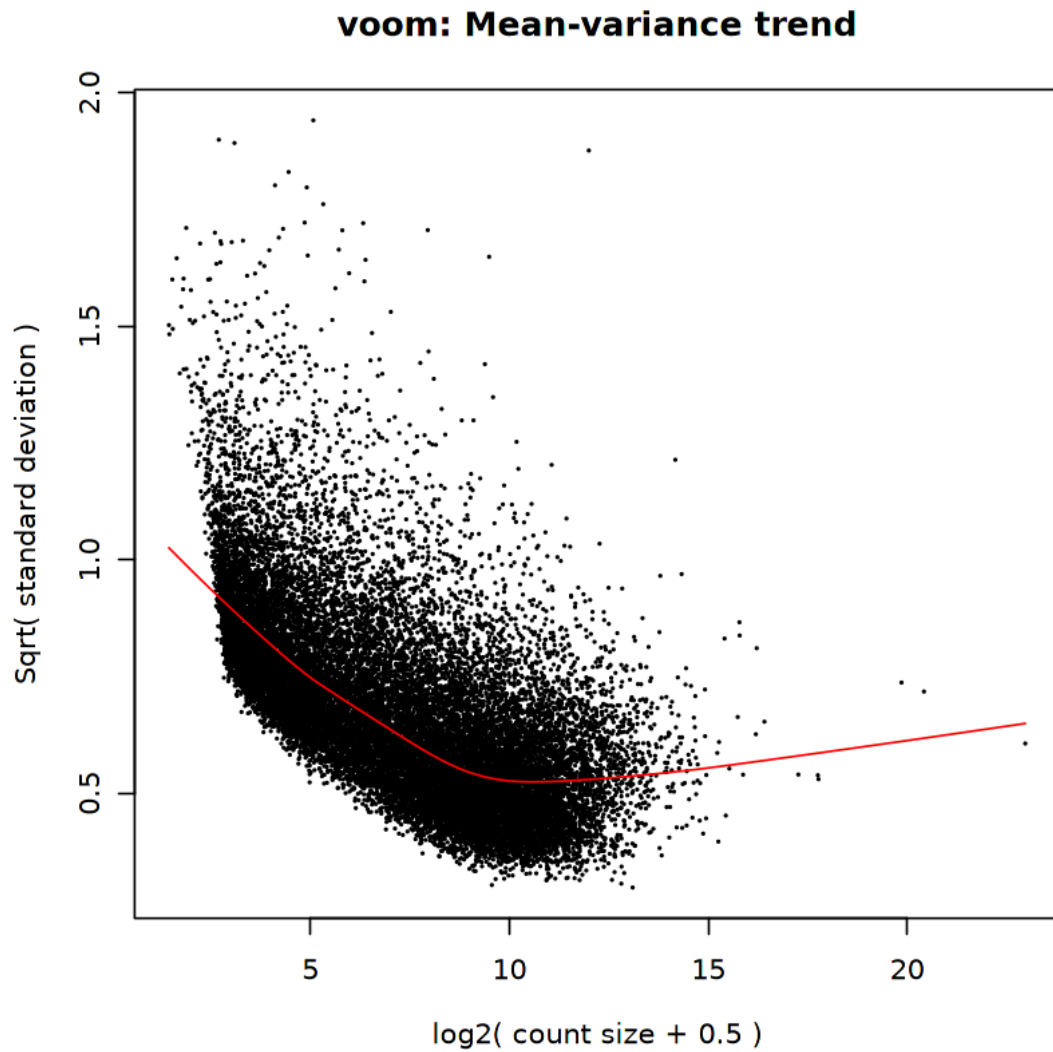
Warning message in if (svobj$sv == 0) {:
"the condition has length > 1 and only the first element will be used"
```

voom: Mean-variance trend



```
[1] "Comparison for: CtrlvsSZ"
[1] "There are: 97 DE features!"
[1] "There are: 22676 features left!"
Number of significant surrogate variables is: 1
Iteration (out of 5 ):1 2 3 4 5

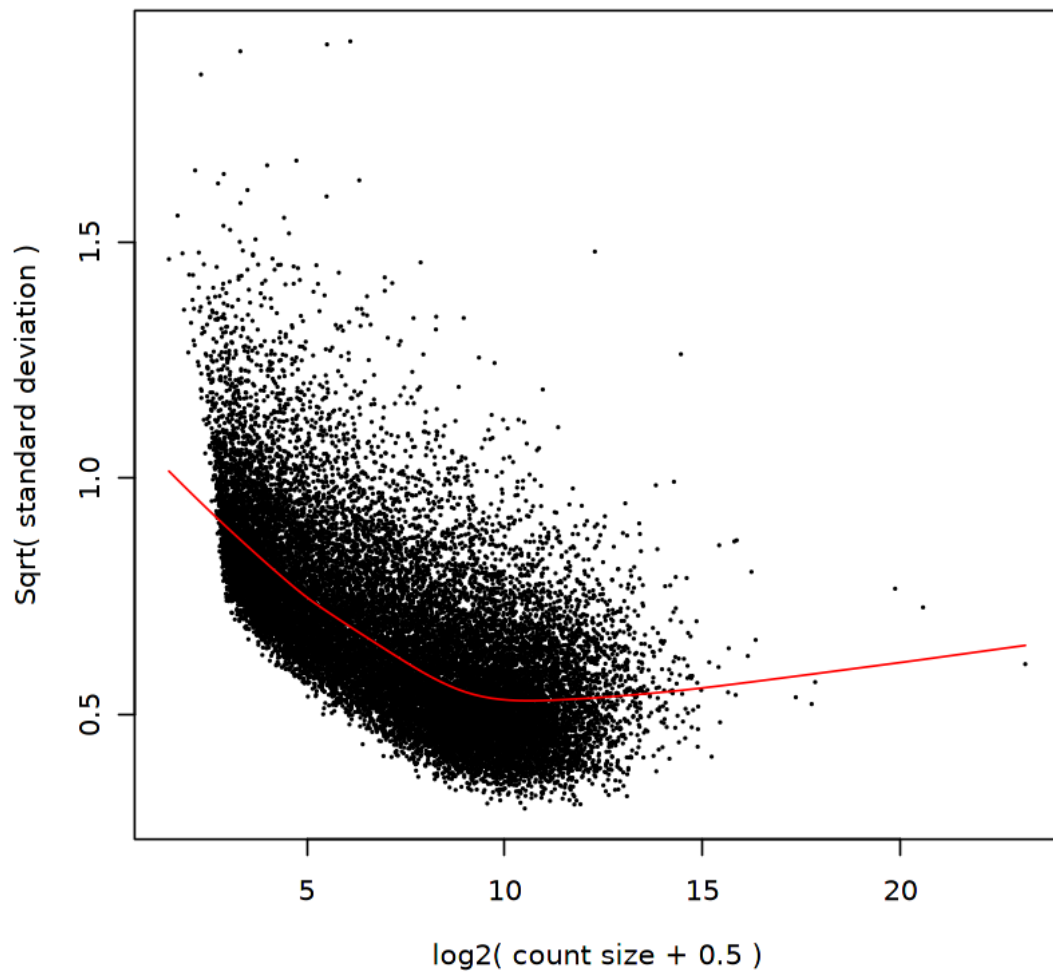
Warning message in if (svobj$sv == 0) {:
"the condition has length > 1 and only the first element will be used"
```

```
[1] "Comparison for: CtrlvsSZ"
[1] "There are: 0 DE features!"
[1] "There are: 22648 features left!"
Number of significant surrogate variables is: 1
Iteration (out of 5 ):1 2 3 4 5

Warning message in if (svobj$sv == 0) {:
"the condition has length > 1 and only the first element will be used"
```

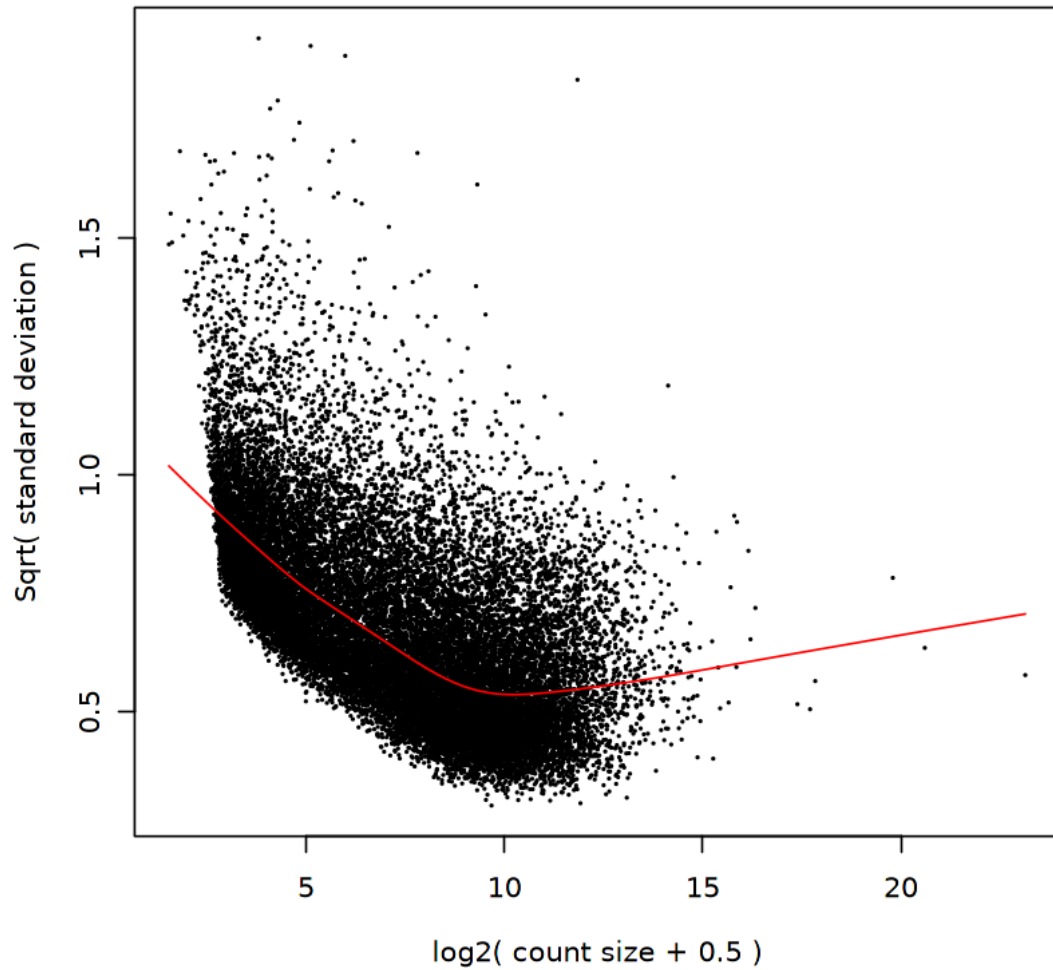
voom: Mean-variance trend



```
[1] "Comparison for: CtrlvsSZ"
[1] "There are: 0 DE features!"
[1] "There are: 22587 features left!"
Number of significant surrogate variables is: 1
Iteration (out of 5 ):1 2 3 4 5

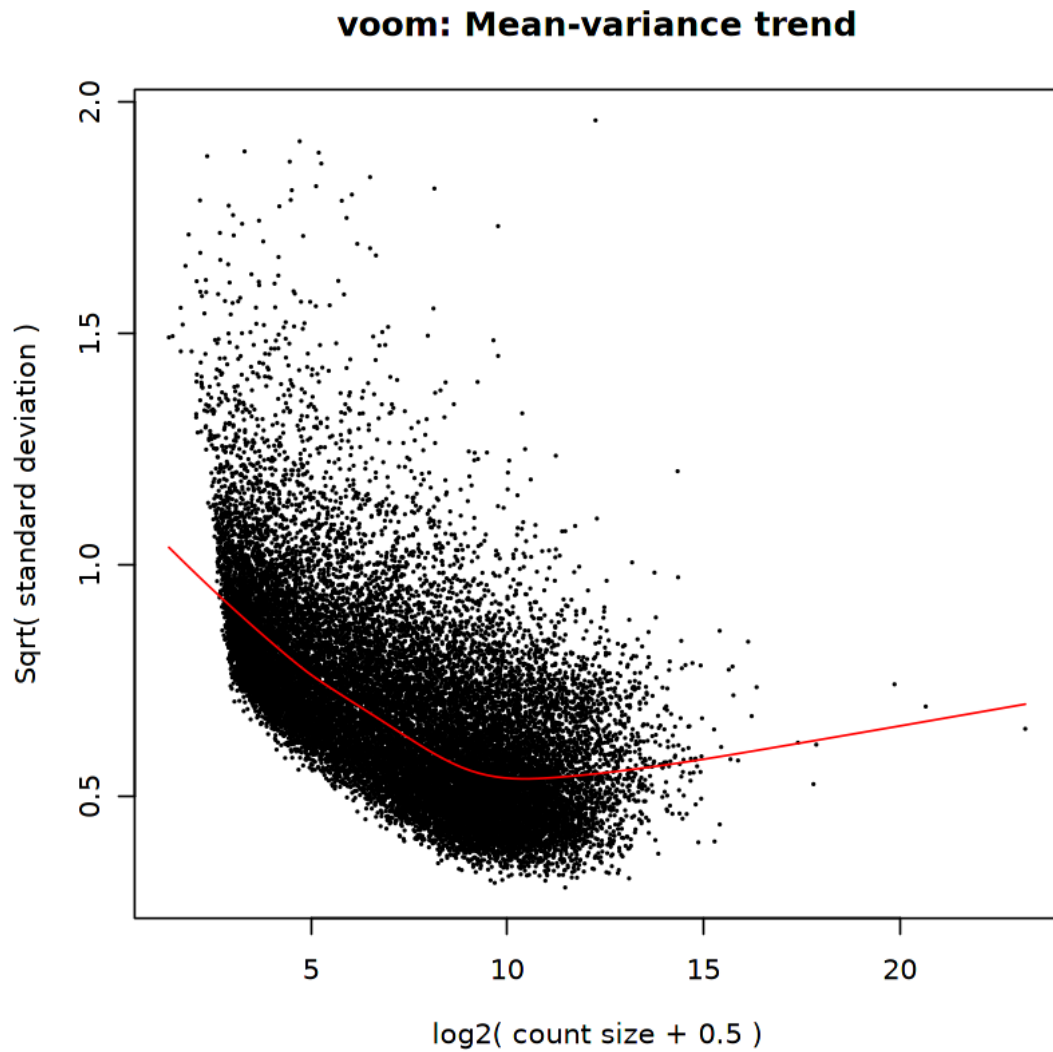
Warning message in if (svobj$sv == 0) {:
"the condition has length > 1 and only the first element will be used"
```

voom: Mean-variance trend



```
[1] "Comparison for: CtrlvsSZ"
[1] "There are: 0 DE features!"
[1] "There are: 22878 features left!"
Number of significant surrogate variables is: 1
Iteration (out of 5 ):1 2 3 4 5

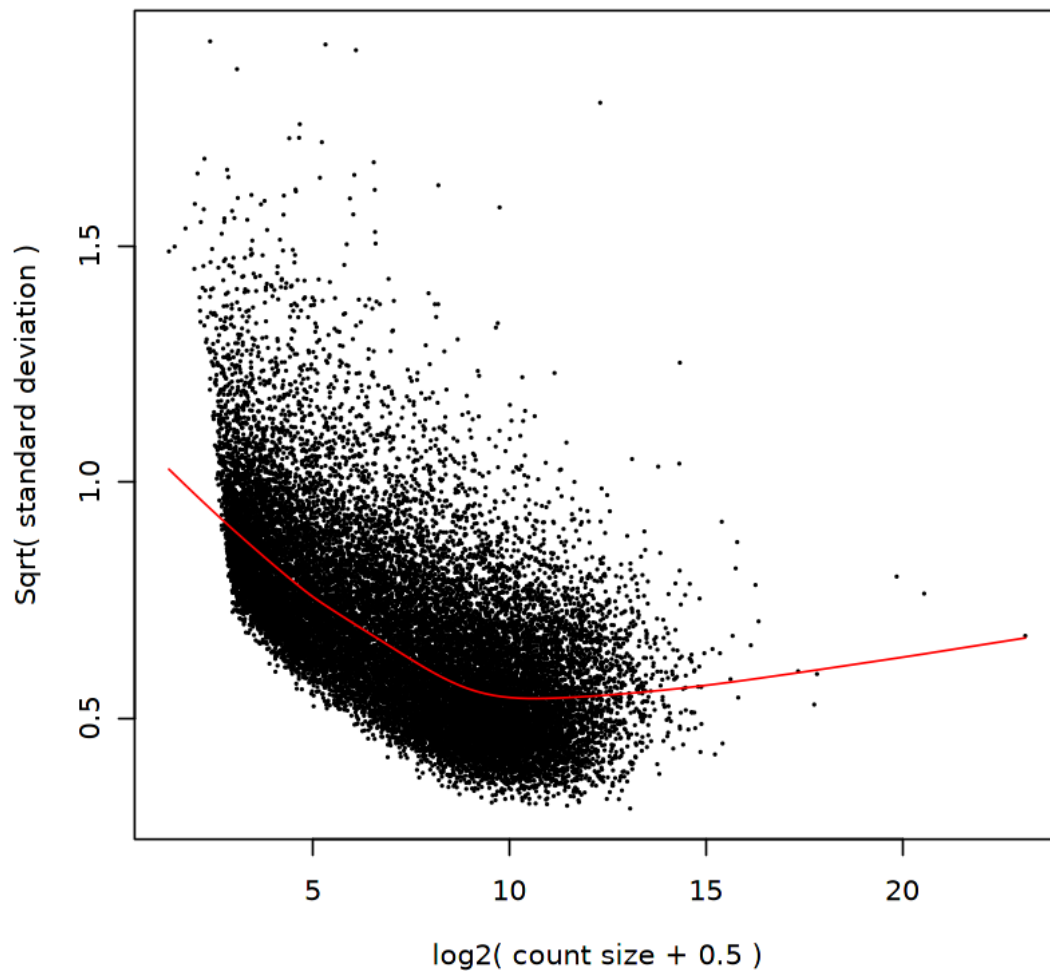
Warning message in if (svobj$sv == 0) {:
"the condition has length > 1 and only the first element will be used"
```



```
[1] "Comparison for: CtrlvsSZ"
[1] "There are: 0 DE features!"
[1] "There are: 22440 features left!"
Number of significant surrogate variables is: 1
Iteration (out of 5 ):1 2 3 4 5

Warning message in if (svobj$sv == 0) {:
"the condition has length > 1 and only the first element will be used"
```

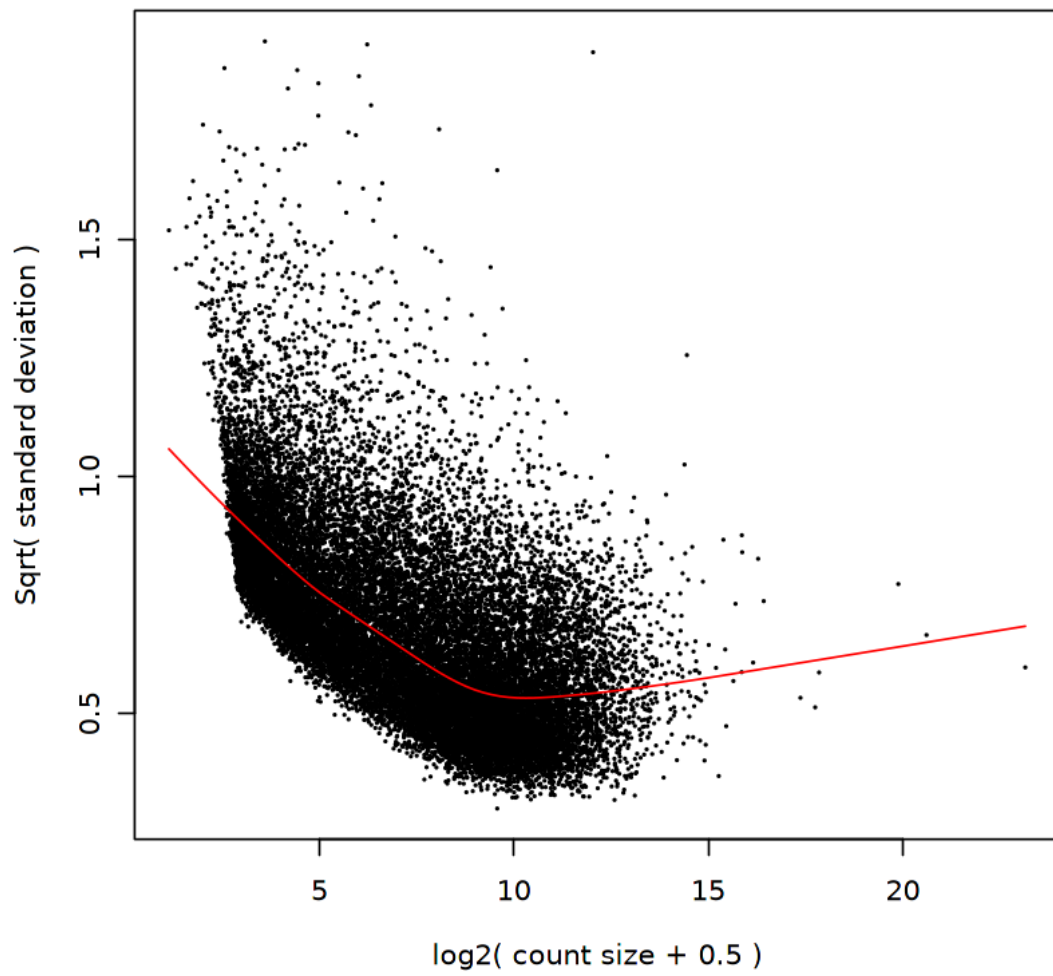
voom: Mean-variance trend



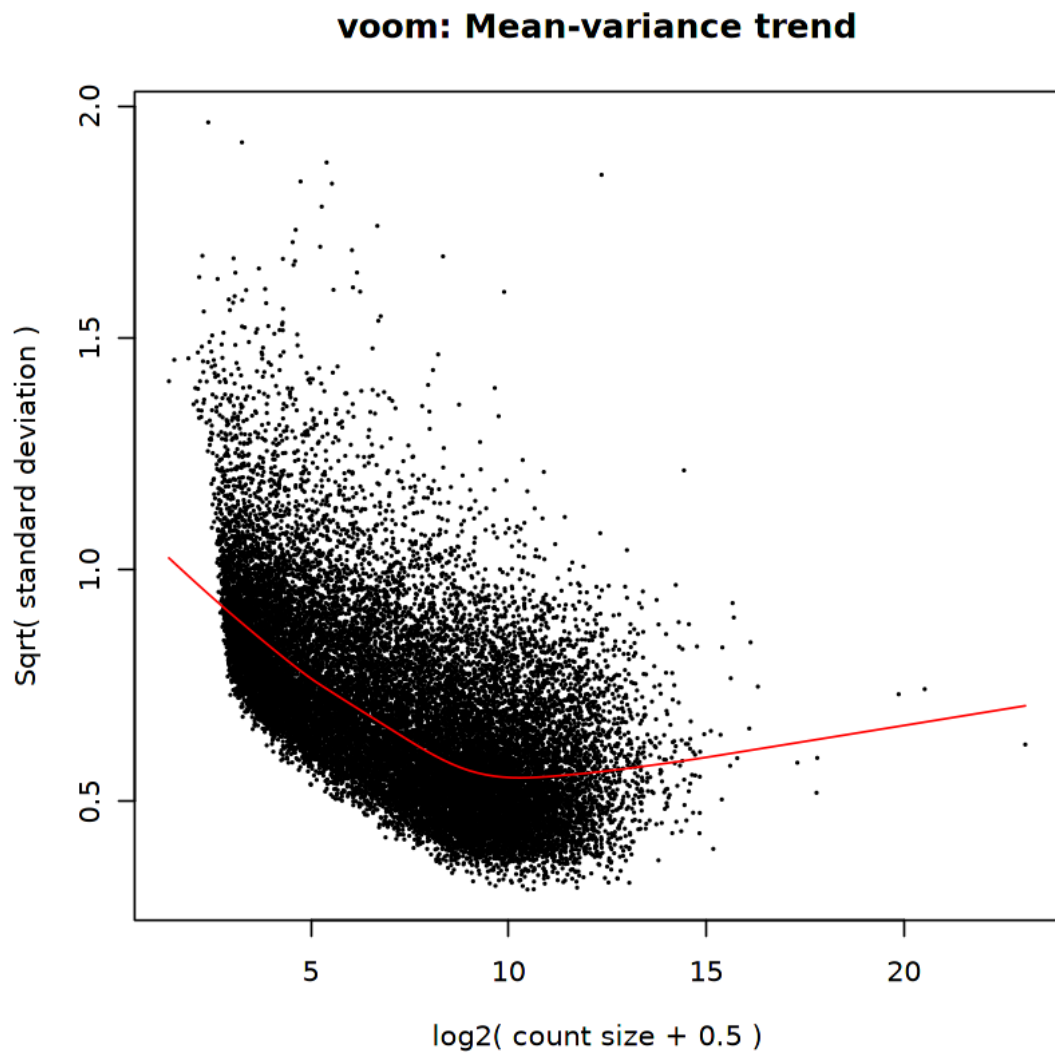
```
[1] "Comparison for: CtrlvsSZ"
[1] "There are: 2 DE features!"
[1] "There are: 23017 features left!"
Number of significant surrogate variables is: 1
Iteration (out of 5 ):1 2 3 4 5

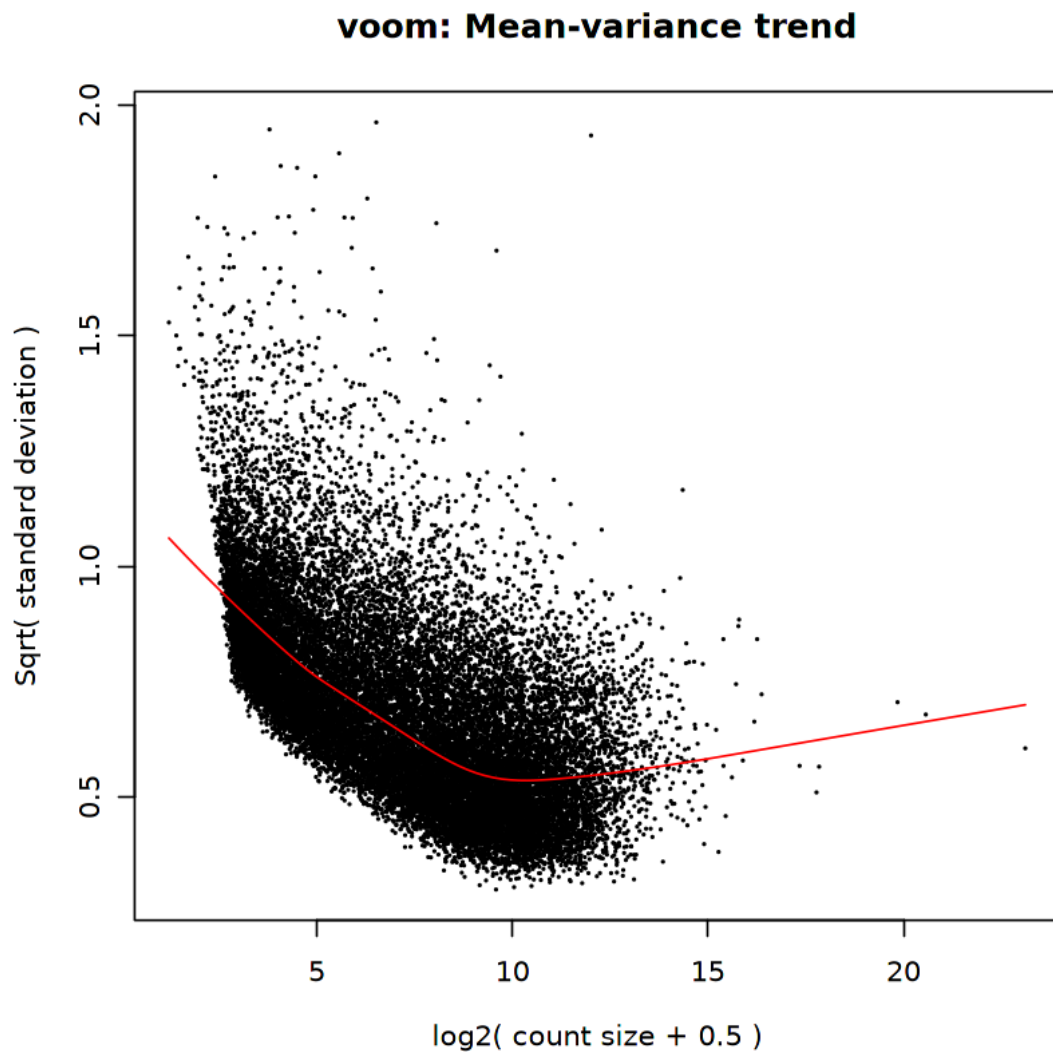
Warning message in if (svobj$sv == 0) {:
"the condition has length > 1 and only the first element will be used"
```

voom: Mean-variance trend



```
[1] "Comparison for: CtrlvsSZ"  
[1] "There are: 19 DE features!"
```





1.4 Reproducibility Information

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

```
[1] "2021-08-12 18:54:55 EDT"
```

```
      user  system elapsed  
2518.587  615.440  571.276
```

```
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    2021-08-12

```

Packages

package	* version	date	lib	source
annotate	1.68.0	2020-10-27	[1]	Bioconductor
AnnotationDbi	1.52.0	2020-10-27	[1]	Bioconductor
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
BiocParallel	* 1.24.1	2020-11-06	[1]	Bioconductor
bit	4.0.4	2020-08-04	[1]	CRAN (R 4.0.2)
bit64	4.0.5	2020-08-30	[1]	CRAN (R 4.0.2)
bitops	1.0-7	2021-04-24	[1]	CRAN (R 4.0.3)
blob	1.2.1	2020-01-20	[1]	CRAN (R 4.0.2)
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)
genefilter	* 1.72.1	2021-01-21	[1]	Bioconductor
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)
mgcv	* 1.8-33	2020-08-27	[2]	CRAN (R 4.0.3)
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)
nlme	* 3.1-152	2021-02-04	[1]	CRAN (R 4.0.3)
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)
RSQLite	2.2.7	2021-04-22	[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	1.7.3	2021-07-16	[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
survival	3.2-7	2020-09-28	[2]	CRAN (R 4.0.3)

sva	* 3.38.0	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)
XML	3.99-0.6	2021-03-16	[1]	CRAN (R 4.0.3)
xml2	1.3.2	2020-04-23	[1]	CRAN (R 4.0.2)
xtable	1.8-4	2019-04-21	[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