

Appendix 2

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```
library("dplyr")  # for arrange() function

##
## Attaching package: 'dplyr'
## The following objects are masked from 'package:stats':
##
##     filter, lag
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
library(limma)      # main limma package
library(edgeR)       # we need the TMM function from edgeR
library(ggplot2)
library(pheatmap)
library(tidyverse)

## -- Attaching packages ----- tidyverse 1.3.0 --
## v tibble   3.1.0    v purrr    0.3.4
## v tidyverse 1.1.3    v stringr  1.4.0
## v readr    1.4.0    vforcats  0.5.1

## -- Conflicts ----- tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()   masks stats::lag()
```

Differentially expressed gene analysis

count matrix:

```
srr <- read.table('srrlist.txt')
head(srr)

##
##          V1
## 1 SRR11412215
## 2 SRR11412216
## 3 SRR11412217
## 4 SRR11412218
## 5 SRR11412219
## 6 SRR11412220

counts_col <- c('gene.id', srr$V1)
counts_all <- read.csv('counts_all.txt', header = F, sep = '\t')
```

```

colnames(counts_all) <- counts_col
head(counts_all[,1:6])

##      gene.id SRR11412215 SRR11412216 SRR11412217 SRR11412218 SRR11412219
## 1     A1BG        2         4         2         2         3
## 2 A1BG-AS1       1         2         4         1         2
## 3     A1CF        0         0         0         0         1
## 4     A2M          0         1         0         1         1
## 5 A2M-AS1        1         0         2         0         1
## 6     A2ML1      365       386       395       387       325

tail(counts_all[,1:6])

##                               gene.id SRR11412215 SRR11412216 SRR11412217 SRR11412218
## 28265                      ZZZ3      149       157       142       140
## 28266      __no_feature    904187     885737     916589     897453
## 28267      __ambiguous    72864      71416      74096      73747
## 28268      __too_low_aQual     0          0          0          0
## 28269      __not_aligned    68189      65390      52874      51269
## 28270 __alignment_not_unique 227126     222129     229765     225689
##     SRR11412219
## 28265      125
## 28266      938896
## 28267      70354
## 28268      0
## 28269      84720
## 28270      222190

counts_all$Series1_NHBE_Mock_1 = rowSums(counts_all[,2:5])
counts_all$Series1_NHBE_Mock_2 = rowSums(counts_all[,6:9])
counts_all$Series1_NHBE_Mock_3 = rowSums(counts_all[,10:13])

counts_all$Series1_NHBE_SARS.CoV.2_1 = rowSums(counts_all[,14:17])
counts_all$Series1_NHBE_SARS.CoV.2_2 = rowSums(counts_all[,18:21])
counts_all$Series1_NHBE_SARS.CoV.2_3 = rowSums(counts_all[,22:25])

counts_all$Series2_A549_Mock_1 =rowSums(counts_all[,26:29])
counts_all$Series2_A549_Mock_2 =rowSums(counts_all[,30:33])
counts_all$Series2_A549_Mock_3 =rowSums(counts_all[,34:37])

counts_all$Series2_A549_SARS.CoV.2_1=rowSums(counts_all[,38:41])
counts_all$Series2_A549_SARS.CoV.2_2=rowSums(counts_all[,42:45])
counts_all$Series2_A549_SARS.CoV.2_3=rowSums(counts_all[,46:49])

counts_all$Series4_A549_Mock_1=rowSums(counts_all[,50:53])
counts_all$Series4_A549_Mock_2=rowSums(counts_all[,54:57])

counts_all$Series4_A549_IAV_1=rowSums(counts_all[,58:61])
counts_all$Series4_A549_IAV_2=rowSums(counts_all[,62:65])

counts_all$Series5_A549_Mock_1=counts_all$SRR11517674
counts_all$Series5_A549_Mock_2=counts_all$SRR11517675
counts_all$Series5_A549_Mock_3=counts_all$SRR11517676

counts_all$Series5_A549_SARS.CoV.2_1=counts_all$SRR11517677

```

```

counts_all$Series5_A549_SARS.CoV.2_2=counts_all$SRR11517678
counts_all$Series5_A549_SARS.CoV.2_3=counts_all$SRR11517679

counts_all$Series6_A549.ACE2_Mock_1=counts_all$SRR11517680
counts_all$Series6_A549.ACE2_Mock_2=counts_all$SRR11517681
counts_all$Series6_A549.ACE2_Mock_3=counts_all$SRR11517682

counts_all$Series6_A549.ACE2_SARS.CoV.2_1=counts_all$SRR11517741
counts_all$Series6_A549.ACE2_SARS.CoV.2_2=counts_all$SRR11517742
counts_all$Series6_A549.ACE2_SARS.CoV.2_3=counts_all$SRR11517743

counts_all$Series7_Calu3_Mock_1=counts_all$SRR11517744
counts_all$Series7_Calu3_Mock_2=counts_all$SRR11517745
counts_all$Series7_Calu3_Mock_3=counts_all$SRR11517746

counts_all$Series7_Calu3_SARS.CoV.2_1=counts_all$SRR11517747
counts_all$Series7_Calu3_SARS.CoV.2_2=counts_all$SRR11517748
counts_all$Series7_Calu3_SARS.CoV.2_3=counts_all$SRR11517749

counts_all$Series9_NHBE_Mock_1=rowSums(counts_all[,84:87])
counts_all$Series9_NHBE_Mock_2=rowSums(counts_all[,88:91])
counts_all$Series9_NHBE_Mock_3=rowSums(counts_all[,92:95])
counts_all$Series9_NHBE_Mock_4=rowSums(counts_all[,96:99])

counts_all$Series9_NHBE_IAV_1=rowSums(counts_all[,100:103])
counts_all$Series9_NHBE_IAV_2=rowSums(counts_all[,104:107])
counts_all$Series9_NHBE_IAV_3=rowSums(counts_all[,108:111])
counts_all$Series9_NHBE_IAV_4=rowSums(counts_all[,112:115])

counts_all$Series16_A549.ACE2_Mock_1=rowSums(counts_all[,116:119])
counts_all$Series16_A549.ACE2_Mock_2=rowSums(counts_all[,120:123])
counts_all$Series16_A549.ACE2_Mock_3=rowSums(counts_all[,124:127])
counts_all$Series16_A549.ACE2_SARS.CoV.2_1=rowSums(counts_all[,128:131])
counts_all$Series16_A549.ACE2_SARS.CoV.2_2=rowSums(counts_all[,132:135])
counts_all$Series16_A549.ACE2_SARS.CoV.2_3=rowSums(counts_all[,136:139])

a<-counts_all[,c(1,140:187)]
a1<-data.frame(gene=a$gene.id,
                 Series1_NHBE_Mock_1=a$Series1_NHBE_Mock_1,
                 Series1_NHBE_Mock_2=a$Series1_NHBE_Mock_2,
                 Series1_NHBE_Mock_3=a$Series1_NHBE_Mock_3,
                 Series1_NHBE_SARS.CoV.2_1=a$Series1_NHBE_SARS.CoV.2_1,
                 Series1_NHBE_SARS.CoV.2_2=a$Series1_NHBE_SARS.CoV.2_2,
                 Series1_NHBE_SARS.CoV.2_3=a$Series1_NHBE_SARS.CoV.2_3,
                 Series2_A549_Mock_1=a$Series2_A549_Mock_1,
                 Series2_A549_Mock_2=a$Series2_A549_Mock_2,
                 Series2_A549_Mock_3=a$Series2_A549_Mock_3,
                 Series2_A549_SARS.CoV.2_1=a$Series2_A549_SARS.CoV.2_1,
                 Series2_A549_SARS.CoV.2_2=a$Series2_A549_SARS.CoV.2_2,
                 Series2_A549_SARS.CoV.2_3=a$Series2_A549_SARS.CoV.2_3,

```

```

Series4_A549_Mock_1=a$Series4_A549_Mock_1,
Series4_A549_Mock_2=a$Series4_A549_Mock_2,
Series4_A549_IAV_1=a$Series4_A549_IAV_1,
Series4_A549_IAV_2=a$Series4_A549_IAV_2,
Series5_A549_Mock_1=a$Series5_A549_Mock_1,
Series5_A549_Mock_2=a$Series5_A549_Mock_2,
Series5_A549_Mock_3=a$Series5_A549_Mock_3,
Series5_A549_SARS.CoV.2_1=a$Series5_A549_SARS.CoV.2_1,
Series5_A549_SARS.CoV.2_2=a$Series5_A549_SARS.CoV.2_2,
Series5_A549_SARS.CoV.2_3=a$Series5_A549_SARS.CoV.2_3,
Series6_A549.ACE2_Mock_1=a$Series6_A549.ACE2_Mock_1,
Series6_A549.ACE2_Mock_2=a$Series6_A549.ACE2_Mock_2,
Series6_A549.ACE2_Mock_3=a$Series6_A549.ACE2_Mock_3,
Series6_A549.ACE2_SARS.CoV.2_1=a$Series6_A549.ACE2_SARS.CoV.2_1,
Series6_A549.ACE2_SARS.CoV.2_2=a$Series6_A549.ACE2_SARS.CoV.2_2,
Series6_A549.ACE2_SARS.CoV.2_3=a$Series6_A549.ACE2_SARS.CoV.2_3,
Series7_Calu3_Mock_1=a$Series7_Calu3_Mock_1,
Series7_Calu3_Mock_2=a$Series7_Calu3_Mock_2,
Series7_Calu3_Mock_3=a$Series7_Calu3_Mock_3,
Series7_Calu3_SARS.CoV.2_1=a$Series7_Calu3_SARS.CoV.2_1,
Series7_Calu3_SARS.CoV.2_2=a$Series7_Calu3_SARS.CoV.2_2,
Series7_Calu3_SARS.CoV.2_3=a$Series7_Calu3_SARS.CoV.2_3,
Series9_NHBE_Mock_1=a$Series9_NHBE_Mock_1,
Series9_NHBE_Mock_2=a$Series9_NHBE_Mock_2,
Series9_NHBE_Mock_3=a$Series9_NHBE_Mock_3,
Series9_NHBE_Mock_4=a$Series9_NHBE_Mock_4,
Series9_NHBE_IAV_1=a$Series9_NHBE_IAV_1,
Series9_NHBE_IAV_2=a$Series9_NHBE_IAV_2,
Series9_NHBE_IAV_3=a$Series9_NHBE_IAV_3,
Series9_NHBE_IAV_4=a$Series9_NHBE_IAV_4,
Series16_A549.ACE2_Mock_1=a$Series16_A549.ACE2_Mock_1,
Series16_A549.ACE2_Mock_2=a$Series16_A549.ACE2_Mock_2,
Series16_A549.ACE2_Mock_3=a$Series16_A549.ACE2_Mock_3,
Series16_A549.ACE2_SARS.CoV.2_1=a$Series16_A549.ACE2_SARS.CoV.2_1,
Series16_A549.ACE2_SARS.CoV.2_2=a$Series16_A549.ACE2_SARS.CoV.2_2,
Series16_A549.ACE2_SARS.CoV.2_3=a$Series16_A549.ACE2_SARS.CoV.2_3)

```

```

s9 <- read.table("IFNB.txt")
s9srr <- read.table("s9list.txt")
colnames(s9)<- c("geneID", s9srr$V1)
rownames(s9) <- s9$geneID
s9<-s9[,-1]
a1$Series9_NHBE_IFNB_4h_1 <- rowSums(s9[,1:4])
a1$Series9_NHBE_IFNB_4h_2 <- rowSums(s9[,5:8])
a1$Series9_NHBE_IFNB_6h_1 <- rowSums(s9[,9:12])
a1$Series9_NHBE_IFNB_6h_2 <- rowSums(s9[,13:16])
a1$Series9_NHBE_IFNB_12h_1 <- rowSums(s9[,17:20])
a1$Series9_NHBE_IFNB_12h_2 <- rowSums(s9[,21:24])

```

```

count.table <- a1[1:28265,-1]
rownames(count.table)<-a1$gene[1:28265]
head(count.table[,1:6])

```

```

##          Series1_NHBE_Mock_1 Series1_NHBE_Mock_2 Series1_NHBE_Mock_3

```

```

## A1BG          10          8          9
## A1BG-AS1      8          7         12
## A1CF          0          1          6
## A2M           2          2          0
## A2M-AS1       3          3          0
## A2ML1        1533      1337      2159
##          Series1_NHBE_SARS.CoV.2_1 Series1_NHBE_SARS.CoV.2_2
## A1BG          8          4
## A1BG-AS1     11         10
## A1CF          0          0
## A2M           1          0
## A2M-AS1       1          0
## A2ML1        1294      1311
##          Series1_NHBE_SARS.CoV.2_3
## A1BG          27
## A1BG-AS1     19
## A1CF          0
## A2M           16
## A2M-AS1      12
## A2ML1        5371

tail(count.table[,1:6])

##          Series1_NHBE_Mock_1 Series1_NHBE_Mock_2 Series1_NHBE_Mock_3
## ZXDC          370          389          452
## ZYG11A        2            3            3
## ZYG11B        345          284          557
## ZYX           2793         2906         3707
## ZZEF1         666          615          880
## ZZZ3          588          519          905
##          Series1_NHBE_SARS.CoV.2_1 Series1_NHBE_SARS.CoV.2_2
## ZXDC          373          354
## ZYG11A        3            2
## ZYG11B        288          256
## ZYX           3026         2275
## ZZEF1         587          585
## ZZZ3          423          506
##          Series1_NHBE_SARS.CoV.2_3
## ZXDC          939
## ZYG11A        5
## ZYG11B        783
## ZYX           7345
## ZZEF1         1660
## ZZZ3          1386

dim(count.table)

## [1] 28265    54
colnames(count.table)

##  [1] "Series1_NHBE_Mock_1"          "Series1_NHBE_Mock_2"
##  [3] "Series1_NHBE_Mock_3"          "Series1_NHBE_SARS.CoV.2_1"
##  [5] "Series1_NHBE_SARS.CoV.2_2"      "Series1_NHBE_SARS.CoV.2_3"
##  [7] "Series2_A549_Mock_1"          "Series2_A549_Mock_2"
##  [9] "Series2_A549_Mock_3"          "Series2_A549_SARS.CoV.2_1"

```

```

## [11] "Series2_A549_SARS.CoV.2_2"
## [13] "Series4_A549_Mock_1"
## [15] "Series4_A549_IAV_1"
## [17] "Series5_A549_Mock_1"
## [19] "Series5_A549_Mock_3"
## [21] "Series5_A549_SARS.CoV.2_2"
## [23] "Series6_A549.ACE2_Mock_1"
## [25] "Series6_A549.ACE2_Mock_3"
## [27] "Series6_A549.ACE2_SARS.CoV.2_2"
## [29] "Series7_Calu3_Mock_1"
## [31] "Series7_Calu3_Mock_3"
## [33] "Series7_Calu3_SARS.CoV.2_2"
## [35] "Series9_NHBE_Mock_1"
## [37] "Series9_NHBE_Mock_3"
## [39] "Series9_NHBE_IAV_1"
## [41] "Series9_NHBE_IAV_3"
## [43] "Series16_A549.ACE2_Mock_1"
## [45] "Series16_A549.ACE2_Mock_3"
## [47] "Series16_A549.ACE2_SARS.CoV.2_2"
## [49] "Series9_NHBE_IFNB_4h_1"
## [51] "Series9_NHBE_IFNB_6h_1"
## [53] "Series9_NHBE_IFNB_12h_1" "Series2_A549_SARS.CoV.2_3"
"Series4_A549_Mock_2"
"Series4_A549_IAV_2"
"Series5_A549_Mock_2"
"Series5_A549_SARS.CoV.2_1"
"Series5_A549_SARS.CoV.2_3"
"Series6_A549.ACE2_Mock_2"
"Series6_A549.ACE2_SARS.CoV.2_1"
"Series6_A549.ACE2_SARS.CoV.2_3"
"Series7_Calu3_Mock_2"
"Series7_Calu3_SARS.CoV.2_1"
"Series7_Calu3_SARS.CoV.2_3"
"Series9_NHBE_Mock_2"
"Series9_NHBE_Mock_4"
"Series9_NHBE_IAV_2"
"Series9_NHBE_IAV_4"
"Series16_A549.ACE2_Mock_2"
"Series16_A549.ACE2_SARS.CoV.2_1"
"Series16_A549.ACE2_SARS.CoV.2_3"
"Series9_NHBE_IFNB_4h_2"
"Series9_NHBE_IFNB_6h_2"
"Series9_NHBE_IFNB_12h_2"

```

Series 1: NHBE_SARS.CoV.2 vs NHBE_Mock

```

new.data <- count.table[,1:6]
Series <- colnames(count.table)[1:6]
Treat <- c(rep("Mock",3),rep("SARS",3))
new.meta <- data.frame(Series = Series, Treat = Treat)
new.meta$Treat <- as.factor(new.meta$Treat)

##### design matrix
new.design <- model.matrix(~ Treat, data = new.meta)
new.design

## (Intercept) TreatSARS
## 1           1      0
## 2           1      0
## 3           1      0
## 4           1      1
## 5           1      1
## 6           1      1
## attr(),"assign")
## [1] 0 1
## attr(),"contrasts")
## attr(),"contrasts")$Treat
## [1] "contr.treatment"

##### filtering
# non-specific filtering
dge <- DGEList(counts = new.data)
counts.filt <- rowSums(cpm(dge) > 1) >=3
# Check what fraction of genes remain
sum(counts.filt)/nrow(dge)

```

```

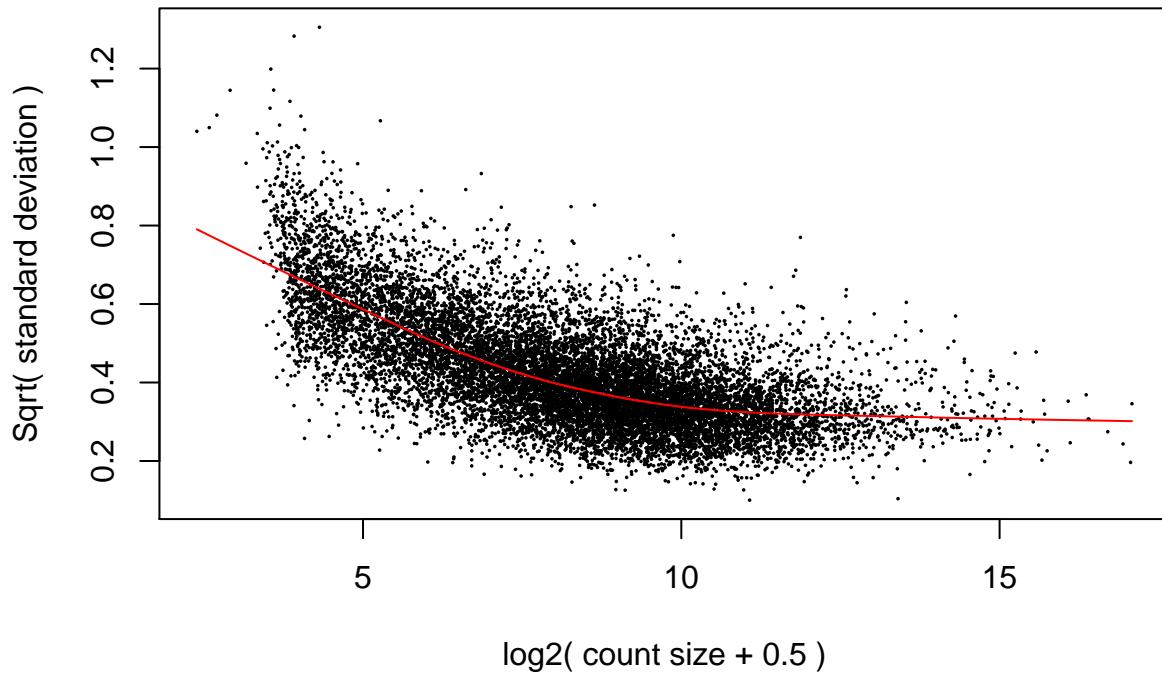
## [1] 0.4644967
# Remove low expressed genes.
dge <- dge[counts.filt,,keep.lib.sizes=T]

##### TMM normalization
dge <- calcNormFactors(dge, method="TMM")

#####
v.1 <- voom(dge,new.design,plot=TRUE)

```

voom: Mean–variance trend

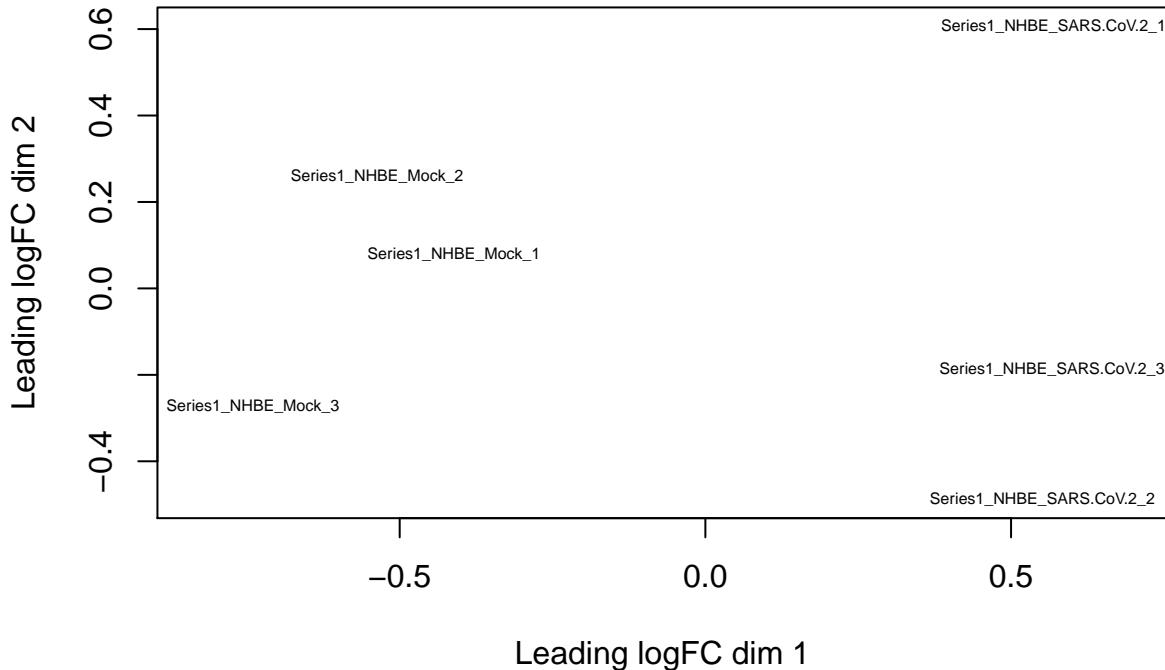


```

##### MDS plot
plotMDS(v.1, main="plotMDS(v)", cex=0.5)

```

plotMDS(v)



```

##### fit linear model
# finding differential expression by fitting linear models
fit <- lmFit(v.1,new.design)
# calculating the statistics
fit2 <- eBayes(fit)
colnames(fit2)

## [1] "(Intercept)" "TreatSARS"

##### list top differentially expressed genes
R.SARS.vs.Mock.nhbe = topTable(fit2, coef="TreatSARS", number=nrow(dge$counts))
dim(R.SARS.vs.Mock.nhbe[R.SARS.vs.Mock.nhbe$adj.P.Val < 0.05,])

## [1] 390   6
head(R.SARS.vs.Mock.nhbe,10)

##          logFC AveExpr      t    P.Value adj.P.Val      B
## CXCL8    2.332051 6.979632 24.82511 1.076737e-12 1.413648e-08 19.33616
## SAA2     2.439011 5.436994 23.07382 2.841142e-12 1.865067e-08 18.15523
## CCL20    3.136193 4.141809 19.05371 3.538681e-11 1.548645e-07 15.35657
## SAA1     2.210900 7.585356 16.83036 1.784647e-10 4.173317e-07 14.52499
## TNFAIP3  1.611034 7.154874 16.74441 1.907221e-10 4.173317e-07 14.45807
## IL36G    2.740718 3.735522 17.32281 1.226932e-10 4.027099e-07 14.21433
## S100A8   1.862112 6.723227 16.13852 3.073833e-10 5.765194e-07 13.98343
## CXCL1    1.416154 6.882296 15.68012 4.459161e-10 5.916137e-07 13.61684
## KRT6B    1.529004 7.478076 15.64185 4.601893e-10 5.916137e-07 13.58377
## INHBA   1.748870 6.544744 15.55069 4.961883e-10 5.916137e-07 13.50989

```

Series 2: A549_SARS-CoV.2 vs A549_Mock (MOI:0.2)

```
new.data <- count.table[,7:12]
Series <- colnames(count.table)[7:12]
Treat <- c(rep("Mock",3),rep("SARS",3))
new.meta <- data.frame(Series = Series, Treat = Treat)
new.meta$Treat <- as.factor(new.meta$Treat)

##### design matrix
new.design <- model.matrix(~ Treat, data = new.meta)
new.design

## (Intercept) TreatSARS
## 1           1      0
## 2           1      0
## 3           1      0
## 4           1      1
## 5           1      1
## 6           1      1
## attr(),"assign")
## [1] 0 1
## attr(),"contrasts")
## attr(),"contrasts")$Treat
## [1] "contr.treatment"

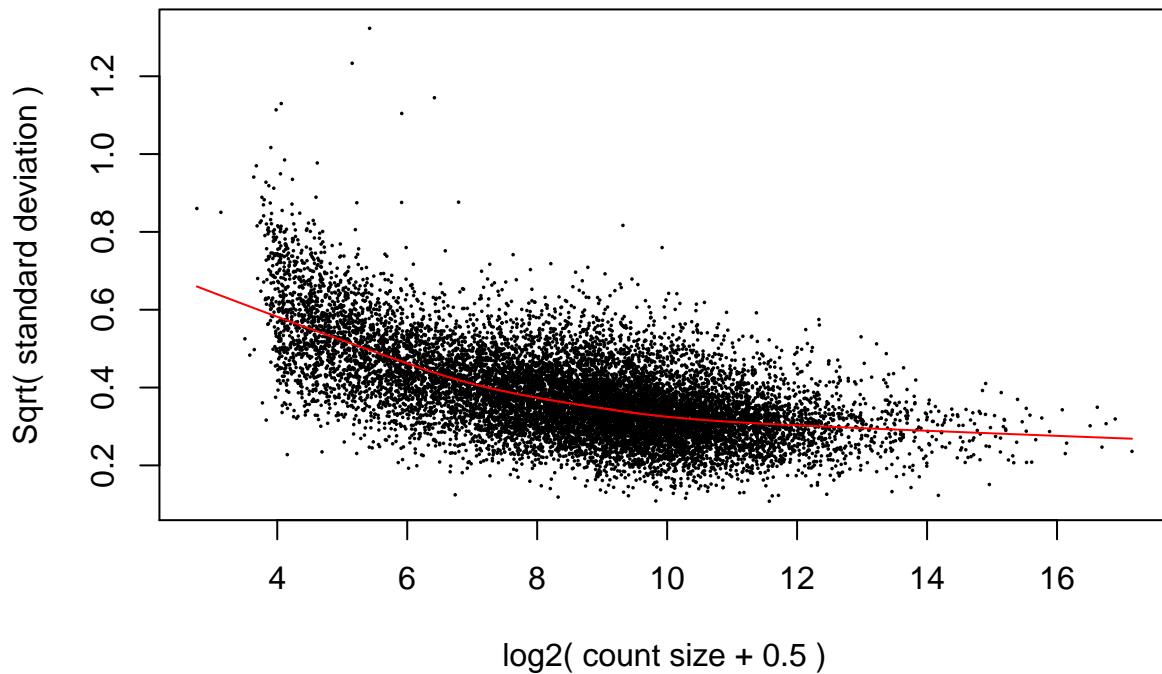
##### filtering
# non-specific filtering
dge <- DGEList(counts = new.data)
counts.filt <- rowSums(cpm(dge) > 1) >=3
# Check what fraction of genes remain
sum(counts.filt)/nrow(dge)

## [1] 0.4727401
# Remove low expressed genes.
dge <- dge[counts.filt,,keep.lib.sizes=T]

##### TMM normalization
dge <- calcNormFactors(dge, method="TMM")

##### VOOM
v.1 <- voom(dge,new.design,plot=TRUE)
```

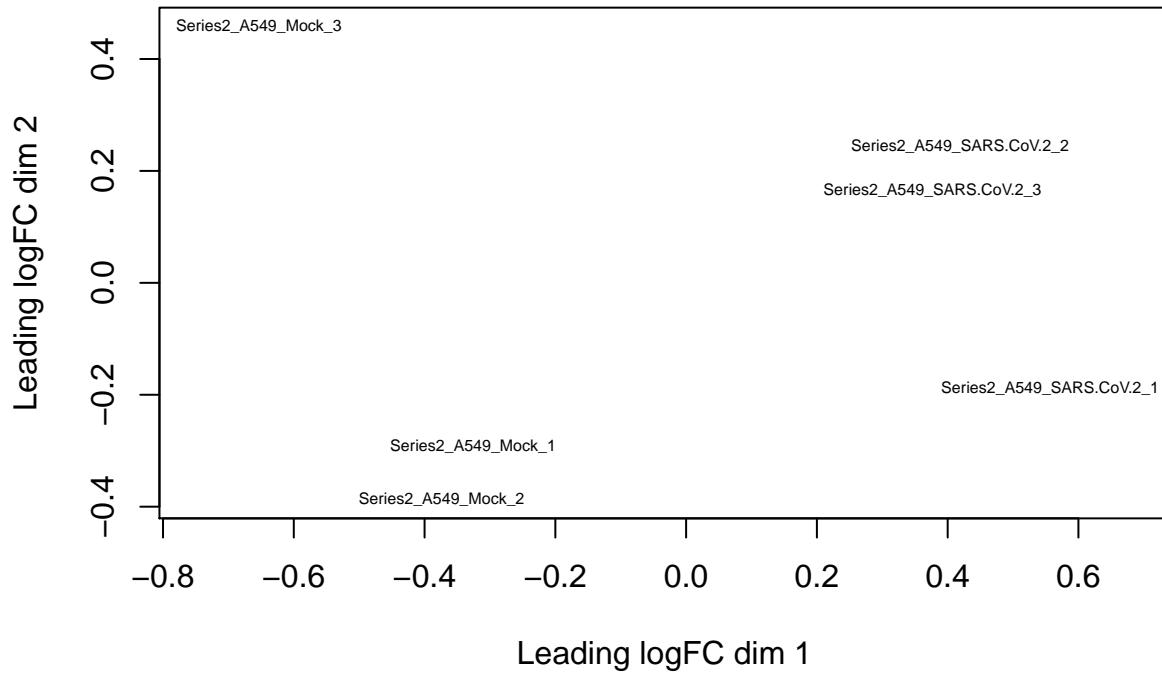
voom: Mean–variance trend



$\log_2(\text{count size} + 0.5)$

```
##### MDS plot
plotMDS(v.1, main="plotMDS(v)", cex=0.5)
```

plotMDS(v)



```
##### fit linear model
# finding differential expression by fitting linear models
```

```

fit <- lmFit(v.1,new.design)
# calculating the statistics
fit2 <- eBayes(fit)
colnames(fit2)

## [1] "(Intercept)" "TreatSARS"

##### list top differentially expressed genes
R.SARS.vs.Mock.A549 = topTable(fit2, coef="TreatSARS", number=nrow(dge$counts))
dim(R.SARS.vs.Mock.A549[R.SARS.vs.Mock.A549$adj.P.Val < 0.05,])

## [1] 154   6
head(R.SARS.vs.Mock.A549,10)

##           logFC AveExpr      t     P.Value adj.P.Val      B
## IFI6    4.333729 5.150743 36.38171 3.586927e-15 4.792852e-11 21.71209
## ISG15   3.747719 3.964616 27.84136 1.403074e-13 9.373938e-10 18.54398
## IRF9    2.164335 5.218737 22.65098 2.321191e-12 6.203150e-09 18.05828
## IFIT1   4.285397 4.001620 24.79026 6.822148e-13 3.038585e-09 17.40587
## PARP9   2.044288 5.240742 20.89395 6.910380e-12 1.538942e-08 17.17668
## OAS1    1.543668 6.685772 20.08712 1.174361e-11 2.241688e-08 16.99939
## OAS3    1.384373 8.121082 18.39786 3.814403e-11 5.859446e-08 15.98615
## MX1     5.007063 3.092945 24.18199 9.561431e-13 3.193996e-09 15.62082
## IRF7    3.148401 3.750230 18.35102 3.946641e-11 5.859446e-08 14.88280
## STAT1   1.318543 8.249644 15.77075 2.954399e-10 3.947668e-07 14.00237

```

Series 4: A549_IAV vs A549_Mock

```

new.data <- count.table[,13:16]
Series <- colnames(count.table)[13:16]
Treat <- c(rep("Mock",2),rep("IAV",2))
new.meta <- data.frame(Series = Series, Treat = Treat)
new.meta$Treat <- as.factor(new.meta$Treat)
new.meta$Treat <- relevel(new.meta$Treat, "Mock")

##### design matrix
new.design <- model.matrix(~ Treat, data = new.meta)
new.design

##   (Intercept) TreatIAV
## 1           1      0
## 2           1      0
## 3           1      1
## 4           1      1
## attr(),"assign")
## [1] 0 1
## attr(),"contrasts")
## attr(),"contrasts")$Treat
## [1] "contr.treatment"

##### filtering
# non-specific filtering
dge <- DGEList(counts = new.data)
counts.filt <- rowSums(cpm(dge) > 1) >=2
# Check what fraction of genes remain

```

```

sum(counts.filt)/nrow(dge)

## [1] 0.4792146

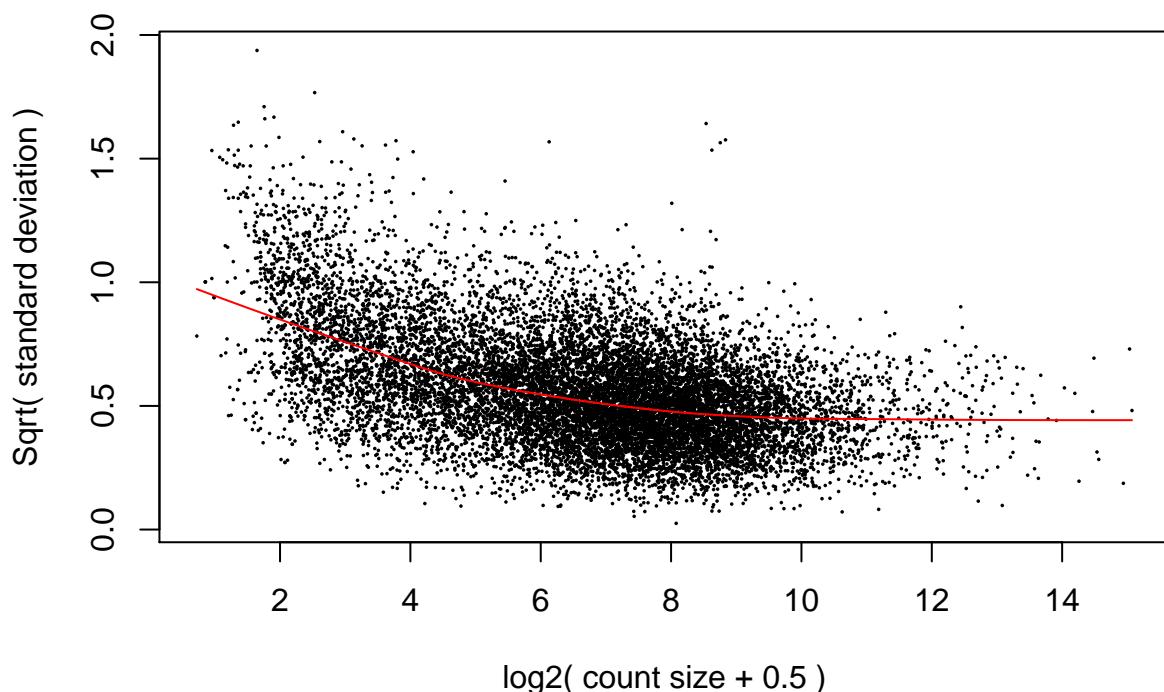
# Remove low expressed genes.
dge <- dge[counts.filt,,keep.lib.sizes=T]

##### TMM normalization
dge <- calcNormFactors(dge, method="TMM")

#### VOOM
v.1 <- voom(dge,new.design,plot=TRUE)

```

voom: Mean–variance trend

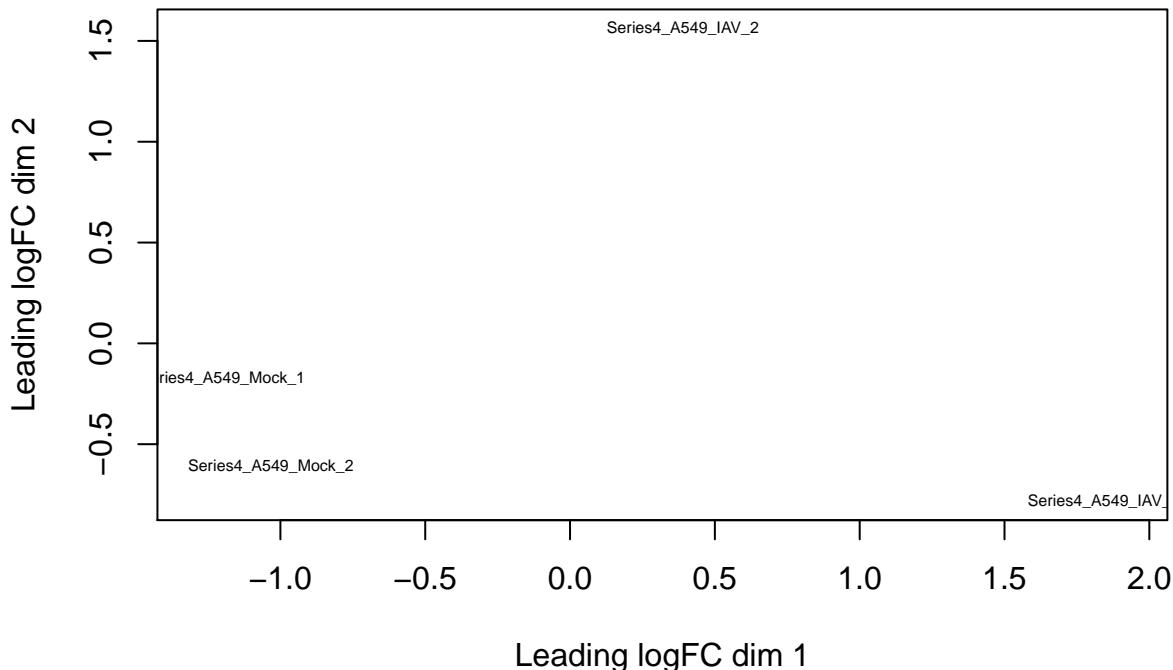


```

##### MDS plot
plotMDS(v.1, main="plotMDS(v)",cex=0.5)

```

plotMDS(v)



```
##### fit linear model
# finding differential expression by fitting linear models
fit <- lmFit(v.1,new.design)
# calculating the statistics
fit2 <- eBayes(fit)
colnames(fit2)

## [1] "(Intercept)" "TreatIAV"
##### list top differentially expressed genes
R.IAV.vs.Mock.A549 = topTable(fit2, coef="TreatIAV", number=nrow(dge$counts))
dim(R.IAV.vs.Mock.A549[R.IAV.vs.Mock.A549$adj.P.Val < 0.05,])

## [1] 0 6
head(R.IAV.vs.Mock.A549,10)

##          logFC    AveExpr         t   P.Value adj.P.Val      B
## FXYD2     -3.269592  3.680135 -8.297803 4.432351e-05 0.1171482 2.367976
## RPL39     -1.722350  6.526089 -7.739126 7.169286e-05 0.1171482 2.230897
## LINC00641  2.275279  3.738314  7.924164 6.095240e-05 0.1171482 2.217770
## MAFK      -1.954294  5.497614 -7.724604 7.262138e-05 0.1171482 2.199946
## ALDOA     -1.672554  5.789219 -7.313182 1.054312e-04 0.1171482 1.856862
## CPLX2     -1.389778 10.263642 -7.030114 1.375751e-04 0.1171482 1.581397
## NEAT1      -1.282762  7.887285 -6.913853 1.538300e-04 0.1171482 1.479469
## LOC100130111 -2.137315  5.038908 -6.844448 1.645471e-04 0.1171482 1.423220
## WBP1      -3.069397  2.284600 -7.168360 1.206862e-04 0.1171482 1.151929
## FBXW10    2.033642  4.001569  6.518085 2.274436e-04 0.1171482 1.096831
```

Series 5: A549_SARS-CoV.2 vs A549_Mock (MOI:2)

```
new.data <- count.table[,17:22]
Series <- colnames(count.table)[17:22]
Treat <- c(rep("Mock",3),rep("SARS",3))
new.meta <- data.frame(Series = Series, Treat = Treat)
new.meta$Treat <- as.factor(new.meta$Treat)
new.meta$Treat <- relevel(new.meta$Treat, "Mock")

##### design matrix
new.design <- model.matrix(~ Treat, data = new.meta)
new.design

##   (Intercept) TreatSARS
## 1           1        0
## 2           1        0
## 3           1        0
## 4           1        1
## 5           1        1
## 6           1        1
## attr(,"assign")
## [1] 0 1
## attr(,"contrasts")
## attr(,"contrasts")$Treat
## [1] "contr.treatment"

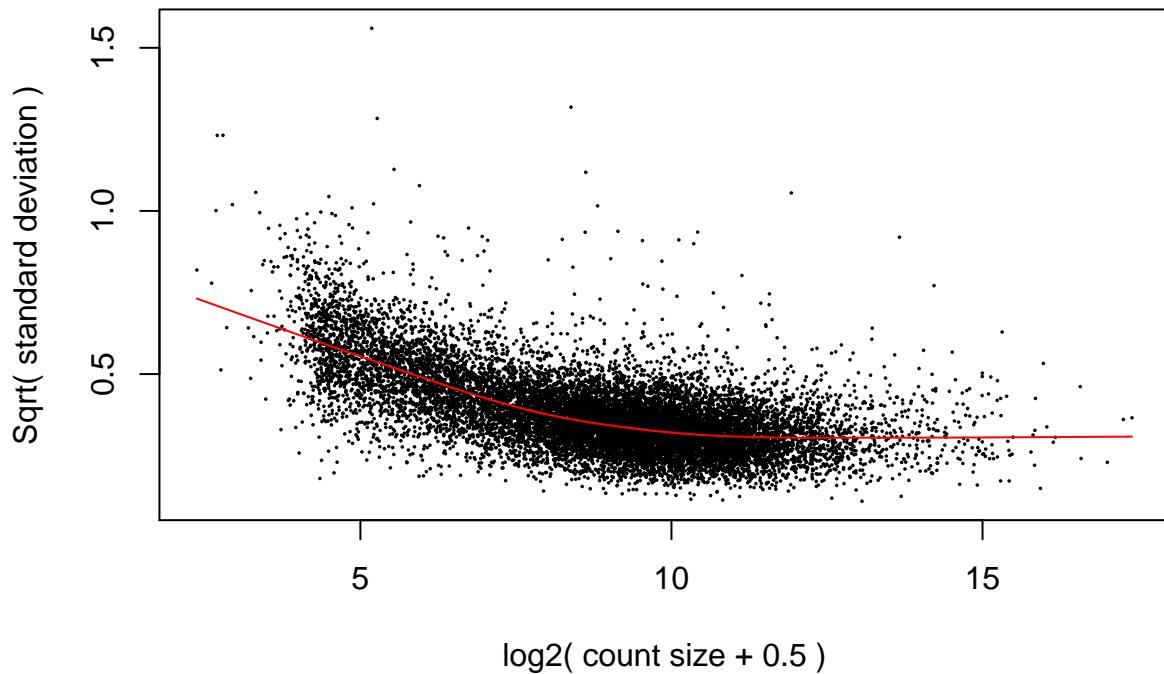
##### filtering
# non-specific filtering
dge <- DGEList(counts = new.data)
counts.filt <- rowSums(cpm(dge) > 1) >=3
# Check what fraction of genes remain
sum(counts.filt)/nrow(dge)

## [1] 0.4832478
# Remove low expressed genes.
dge <- dge[counts.filt,,keep.lib.sizes=T]

##### TMM normalization
dge <- calcNormFactors(dge, method="TMM")

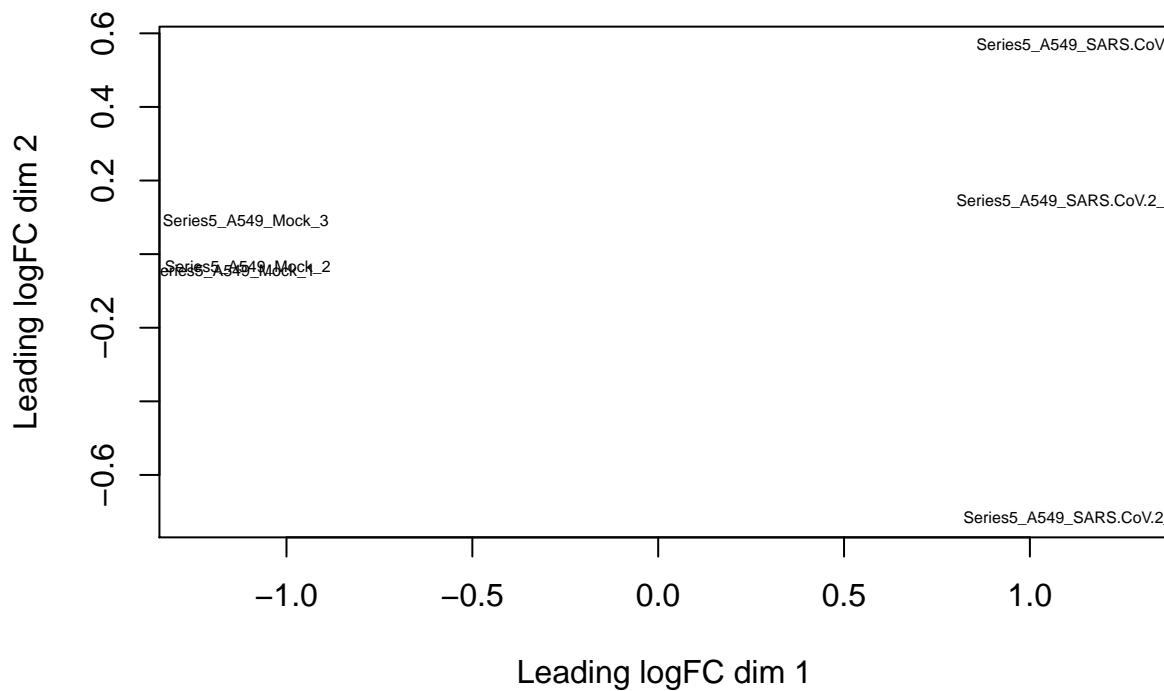
#####
voom <- voom(dge,new.design,plot=TRUE)
```

voom: Mean–variance trend



```
##### MDS plot  
plotMDS(v.1, main="plotMDS(v)", cex=0.5)
```

plotMDS(v)



```
##### fit linear model  
# finding differential expression by fitting linear models
```

```

fit <- lmFit(v.1,new.design)
# calculating the statistics
fit2 <- eBayes(fit)
colnames(fit2)

## [1] "(Intercept)" "TreatSARS"

##### list top differentially expressed genes
R.SARS.vs.Mock.A549.2 = topTable(fit2, coef="TreatSARS", number=nrow(dge$counts))
dim(R.SARS.vs.Mock.A549.2[R.SARS.vs.Mock.A549.2$adj.P.Val < 0.05,])

## [1] 9046     6
head(R.SARS.vs.Mock.A549.2,10)

##           logFC    AveExpr      t    P.Value   adj.P.Val       B
## STC2    4.013168  6.378472  41.63338 1.089796e-14 1.488552e-10 23.57583
## GPX2   -2.779779  8.229357 -35.55407 7.585425e-14 4.714611e-10 22.17594
## FASN   -2.256610  8.052107 -33.65921 1.485321e-13 4.714611e-10 21.55430
## LAMC2   3.696261  7.235400  33.28714 1.702212e-13 4.714611e-10 21.35810
## MCM5   -2.389921  6.476261 -32.75289 2.075642e-13 4.714611e-10 21.18838
## TK1    -2.224272  6.711634 -31.56589 3.262579e-13 4.714611e-10 20.78395
## PEG10  -2.335901  6.881518 -31.55038 3.282272e-13 4.714611e-10 20.78303
## CXCL8   2.398270  6.719238  31.19620 3.768857e-13 4.714611e-10 20.63127
## KRT8   -2.233791 10.641115 -30.95655 4.141982e-13 4.714611e-10 20.58672
## ICAM1   2.788543  6.135482  31.23238 3.715740e-13 4.714611e-10 20.58437

```

Series 6: A549.ACE2_SARS-CoV.2 vs A549.ACE2_Mock (MOI:0.2)

```

new.data <- count.table[,23:28]
Series <- colnames(count.table)[23:28]
Treat <- c(rep("Mock",3),rep("SARS",3))
new.meta <- data.frame(Series = Series, Treat = Treat)
new.meta$Treat <- as.factor(new.meta$Treat)
new.meta$Treat <- relevel(new.meta$Treat, "Mock")

##### design matrix
new.design <- model.matrix(~ Treat, data = new.meta)
new.design

##   (Intercept) TreatSARS
## 1            1        0
## 2            1        0
## 3            1        0
## 4            1        1
## 5            1        1
## 6            1        1
## attr(),"assign")
## [1] 0 1
## attr(),"contrasts")
## attr(),"contrasts")$Treat
## [1] "contr.treatment"

##### filtering
# non-specific filtering
dge <- DGEList(counts = new.data)

```

```

counts.filt <- rowSums(cpm(dge) > 1) >=3
# Check what fraction of genes remain
sum(counts.filt)/nrow(dge)

## [1] 0.485689

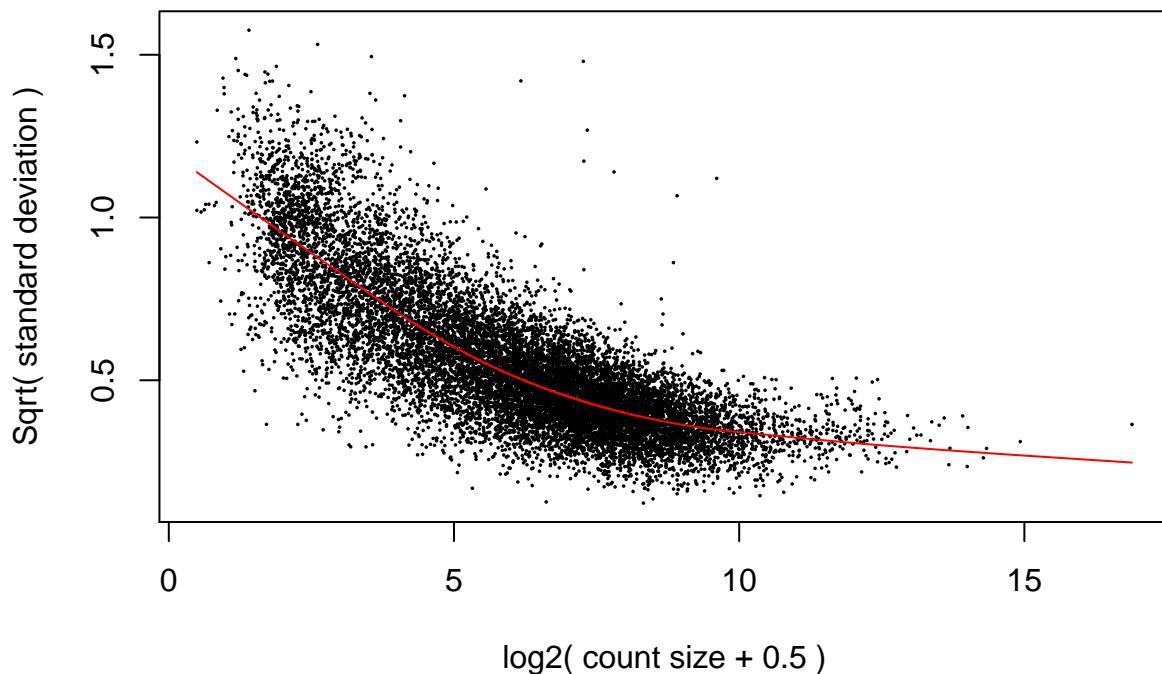
# Remove low expressed genes.
dge <- dge[counts.filt,,keep.lib.sizes=T]

##### TMM normalization
dge <- calcNormFactors(dge, method="TMM")

#### VOOM
v.1 <- voom(dge,new.design,plot=TRUE)

```

voom: Mean-variance trend

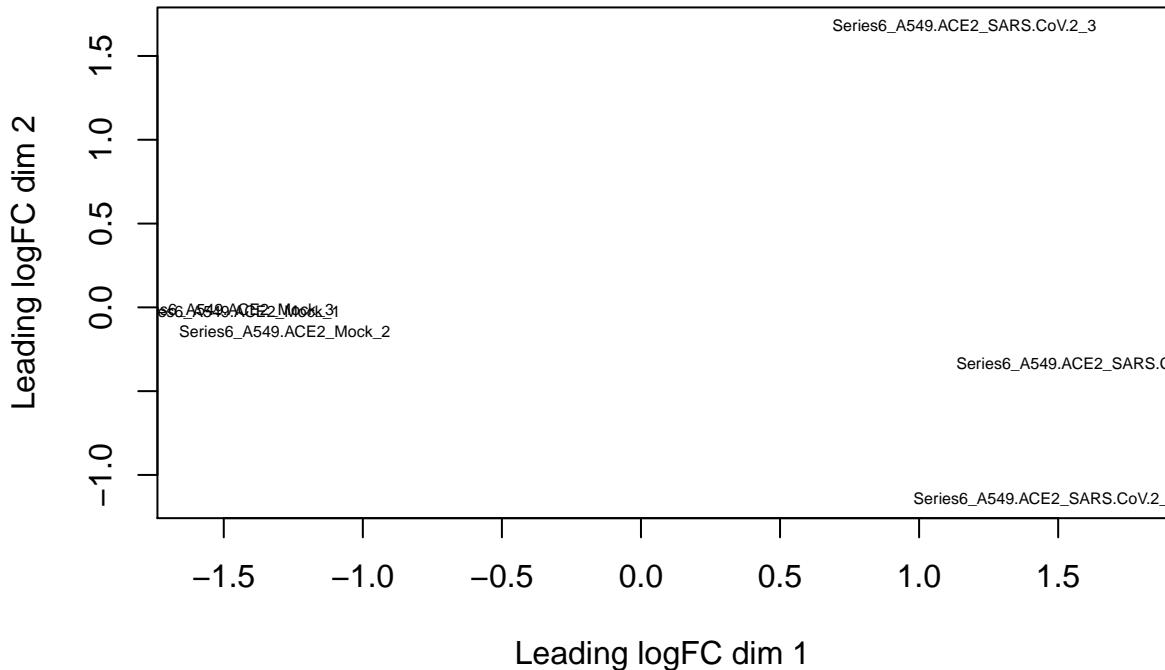


```

##### MDS plot
plotMDS(v.1, main="plotMDS(v)",cex=0.5)

```

plotMDS(v)



```
##### fit linear model
# finding differential expression by fitting linear models
fit <- lmFit(v.1,new.design)
# calculating the statistics
fit2 <- eBayes(fit)
colnames(fit2)

## [1] "(Intercept)" "TreatSARS"

##### list top differentially expressed genes
R.SARS.vs.Mock.A549ACE2 = topTable(fit2, coef="TreatSARS", number=nrow(dge$counts))
dim(R.SARS.vs.Mock.A549ACE2[R.SARS.vs.Mock.A549ACE2$adj.P.Val < 0.05,])

## [1] 5364     6
head(R.SARS.vs.Mock.A549ACE2,10)

##          logFC AveExpr      t    P.Value adj.P.Val      B
## JUNB      3.499349 7.077257 34.04160 3.379191e-23 4.638954e-19 42.88589
## NFKBIA   3.243248 8.512518 30.29346 6.647564e-22 2.281444e-18 40.15891
## CCNL1    2.797940 7.641061 29.92537 9.074512e-22 2.491498e-18 39.80773
## CXCL8    6.877387 5.617665 31.44176 2.575093e-22 1.681408e-18 39.65196
## ARRDC3   3.514599 6.092082 29.40588 1.416684e-21 3.241373e-18 39.11910
## TNFAIP3  3.428361 6.404936 28.65618 2.730323e-21 5.354554e-18 38.57883
## CXCL2    6.858393 5.044990 31.00655 3.674405e-22 1.681408e-18 38.49973
## DDIT3    3.548350 5.823689 28.50425 3.124732e-21 5.362040e-18 38.29563
## PPP1R15A 3.203571 8.003976 27.22431 1.001026e-20 1.526898e-17 37.46763
## IER5     2.296523 7.844013 25.55327 4.955330e-20 6.802677e-17 35.88127
```

Series 7: Calu3_SARS-CoV.2 vs Calu3_Mock

```
new.data <- count.table[, 29:34]
Series <- colnames(count.table)[29:34]
Treat <- c(rep("Mock", 3), rep("SARS", 3))
new.meta <- data.frame(Series = Series, Treat = Treat)
new.meta$Treat <- as.factor(new.meta$Treat)
new.meta$Treat <- relevel(new.meta$Treat, "Mock")

##### design matrix
new.design <- model.matrix(~ Treat, data = new.meta)
new.design

##   (Intercept) TreatSARS
## 1           1        0
## 2           1        0
## 3           1        0
## 4           1        1
## 5           1        1
## 6           1        1
## attr(,"assign")
## [1] 0 1
## attr(,"contrasts")
## attr(,"contrasts")$Treat
## [1] "contr.treatment"

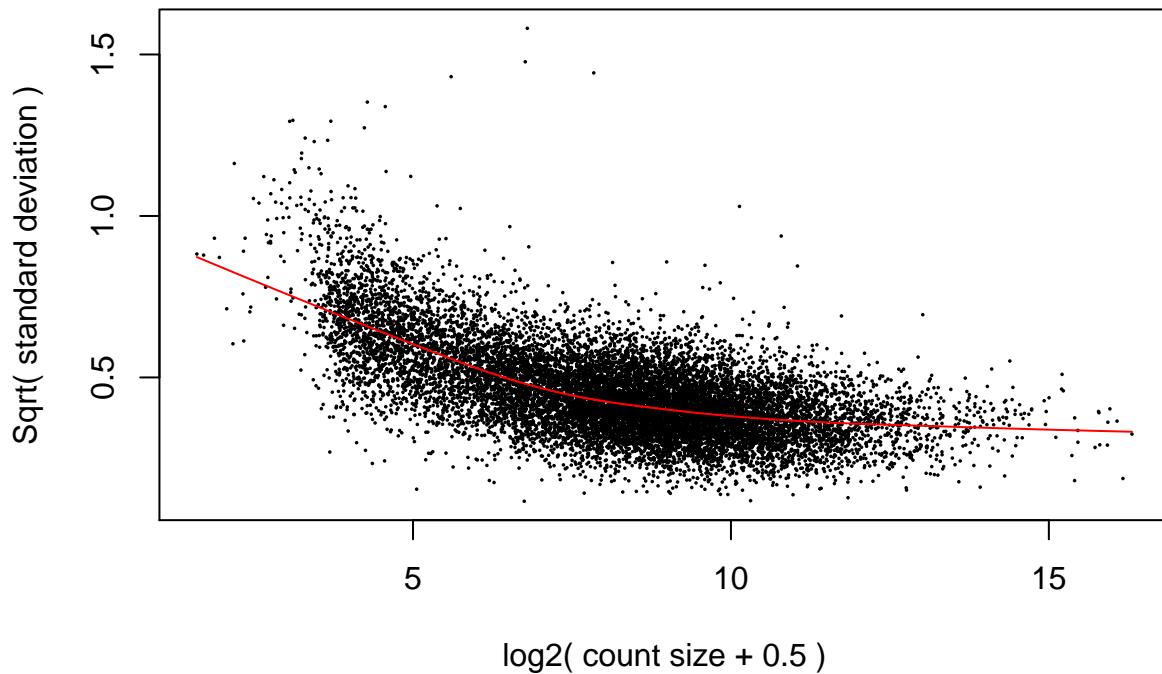
##### filtering
# non-specific filtering
dge <- DGEList(counts = new.data)
counts.filt <- rowSums(cpm(dge) > 1) >= 3
# Check what fraction of genes remain
sum(counts.filt)/nrow(dge)

## [1] 0.4838139
# Remove low expressed genes.
dge <- dge[counts.filt, , keep.lib.sizes=T]

##### TMM normalization
dge <- calcNormFactors(dge, method="TMM")

#####
voom <- voom(dge, new.design, plot=TRUE)
```

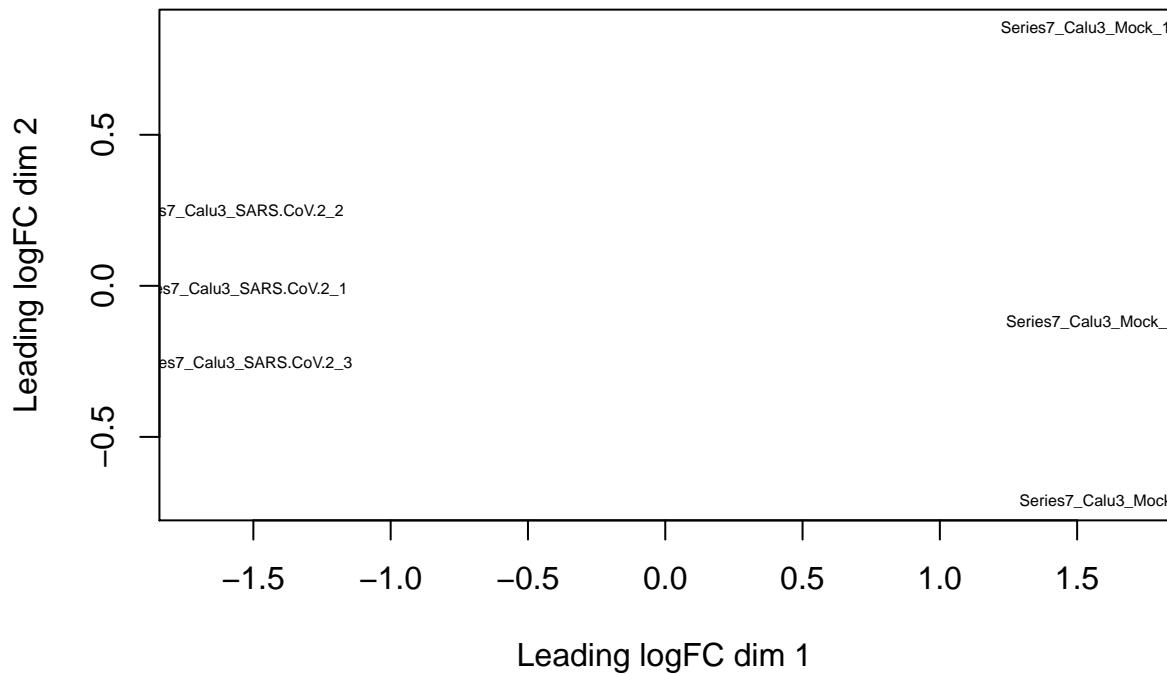
voom: Mean–variance trend



$\log_2(\text{count size} + 0.5)$

```
##### MDS plot  
plotMDS(v.1, main="plotMDS(v)", cex=0.5)
```

plotMDS(v)



```
##### fit linear model  
# finding differential expression by fitting linear models
```

```

fit <- lmFit(v.1,new.design)
# calculating the statistics
fit2 <- eBayses(fit)
colnames(fit2)

## [1] "(Intercept)" "TreatSARS"

##### list top differentially expressed genes
R.SARS.vs.Mock.Calu3 = topTable(fit2, coef="TreatSARS", number=nrow(dge$counts))
dim(R.SARS.vs.Mock.Calu3[R.SARS.vs.Mock.Calu3$adj.P.Val < 0.05,])

## [1] 7337     6
head(R.SARS.vs.Mock.Calu3,10)

##           logFC    AveExpr      t    P.Value   adj.P.Val       B
## IFIT2      5.328834  8.158638 45.13118 2.353311e-17 1.645774e-13 29.94751
## OAS2       4.855717  7.489547 45.06248 2.406982e-17 1.645774e-13 29.86511
## IFIT1      4.520996  7.517083 43.28490 4.366172e-17 1.897718e-13 29.34588
## IFIT3      4.908986  7.021545 42.58799 5.550911e-17 1.897718e-13 29.04745
## RSAD2      5.106672  6.062320 41.78367 7.358777e-17 2.012625e-13 28.57738
## IL1A       5.191369  6.871053 40.73751 1.070486e-16 2.439816e-13 28.41502
## PPP1R15A   3.888958  8.260298 37.79277 3.242103e-16 6.333680e-13 27.54047
## MX1        3.857907  7.716024 36.83086 4.742761e-16 6.876346e-13 27.15351
## TXNIP      3.413315  8.918079 36.22629 6.053997e-16 6.876346e-13 26.96123
## CXCL8      4.279482 10.114352 36.14967 6.245983e-16 6.876346e-13 26.94635

```

Series 9: NHBE_IAV vs NHBE_Mock, NHBE_IFNB vs NHBE_Mock

- NHBE_IAV vs NHBE_Mock
- NHBE_IFNB12h vs NHBE_Mock
- NHBE_IFNB6h vs NHBE_Mock
- NHBE_IFNB4h vs NHBE_Mock

```

new.data <- count.table[,c(35:42,49:54)]
Series <- colnames(count.table)[c(35:42,49:54)]
Treat <- c(rep("Mock",4),rep("IAV",4),rep("IFNB4h",2),rep("IFNB6h",2),rep("IFNB12h",2))
new.meta <- data.frame(Series = Series, Treat = Treat)
new.meta$Treat <- as.factor(new.meta$Treat)
new.meta$Treat <- relevel(new.meta$Treat, "Mock")

##### design matrix
new.design <- model.matrix(~ Treat, data = new.meta)
new.design

##   (Intercept) TreatIAV TreatIFNB12h TreatIFNB4h TreatIFNB6h
## 1           1       0       0       0       0
## 2           1       0       0       0       0
## 3           1       0       0       0       0
## 4           1       0       0       0       0
## 5           1       1       0       0       0
## 6           1       1       0       0       0
## 7           1       1       0       0       0
## 8           1       1       0       0       0
## 9           1       0       0       1       0
## 10          1       0       0       1       0

```

```

## 11      1      0      0      0      1
## 12      1      0      0      0      1
## 13      1      0      1      0      0
## 14      1      0      1      0      0
## attr(,"assign")
## [1] 0 1 1 1 1
## attr(,"contrasts")
## attr(,"contrasts")$Treat
## [1] "contr.treatment"

##### filtering
# non-specific filtering
dge <- DGEList(counts = new.data)
counts.filt <- rowSums(cpm(dge) > 1) >=3
# Check what fraction of genes remain
sum(counts.filt)/nrow(dge)

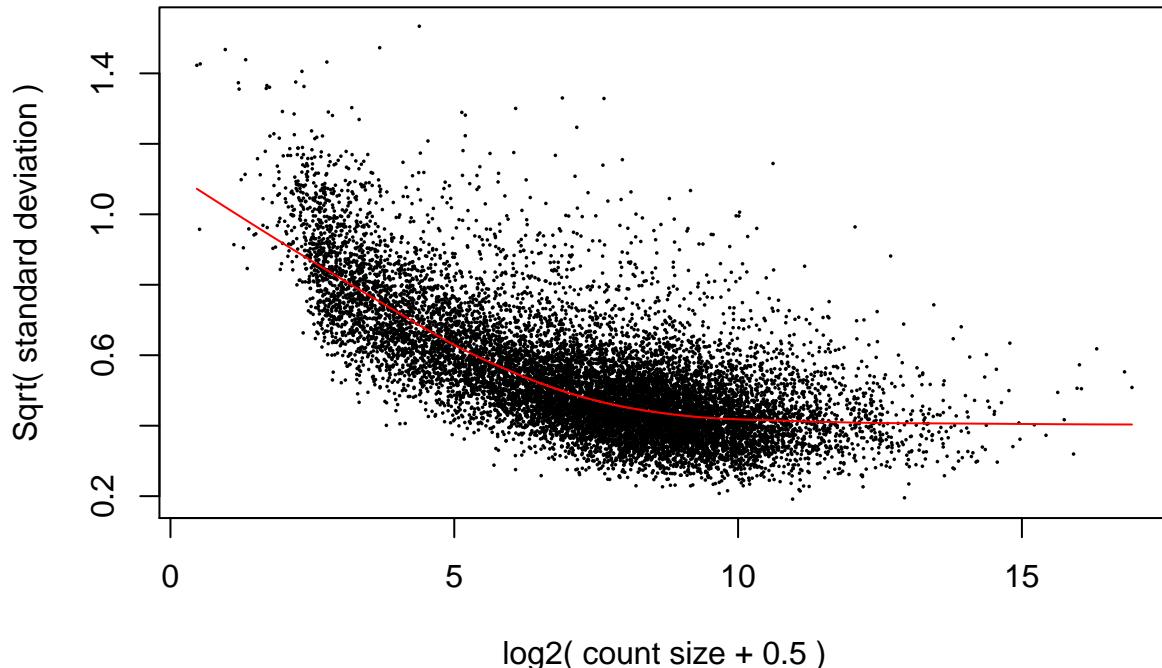
## [1] 0.4741907
# Remove low expressed genes.
dge <- dge[counts.filt,,keep.lib.sizes=T]

##### TMM normalization
dge <- calcNormFactors(dge, method="TMM")

#### VOOM
v.1 <- voom(dge,new.design,plot=TRUE)

```

voom: Mean–variance trend

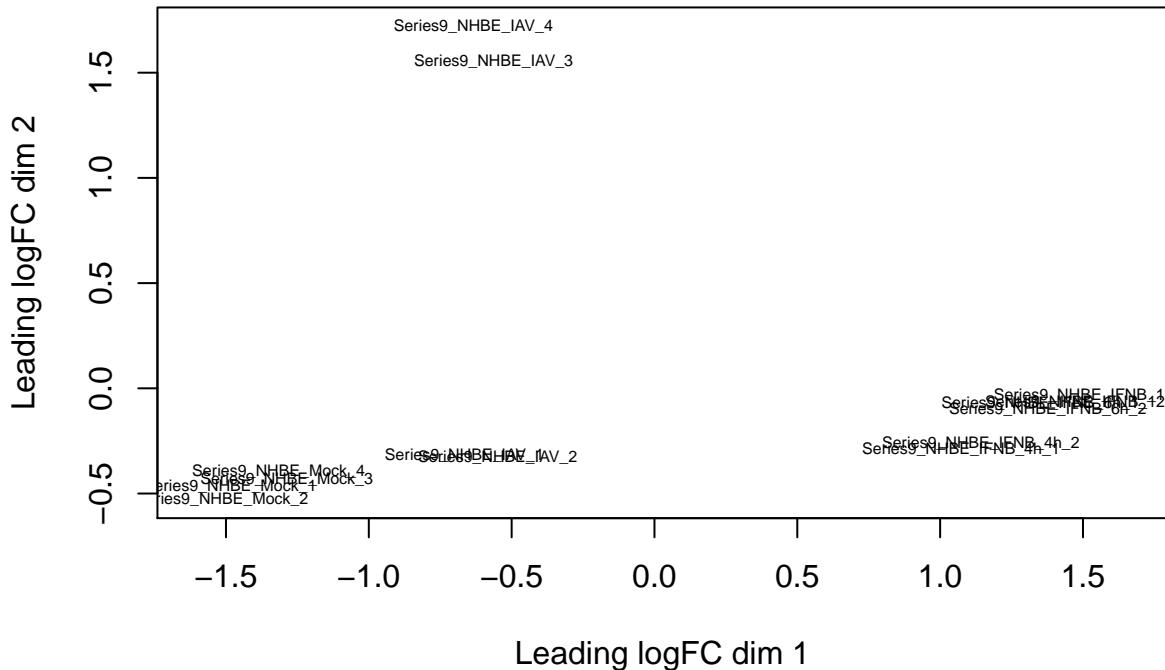


```

##### MDS plot
plotMDS(v.1, main="plotMDS(v)",cex=0.5)

```

plotMDS(v)



```

##### fit linear model
# finding differential expression by fitting linear models
fit <- lmFit(v.1,new.design)
# calculating the statistics
fit2 <- eBayes(fit)
colnames(fit2)

## [1] "(Intercept)" "TreatIAV"      "TreatIFNB12h" "TreatIFNB4h"   "TreatIFNB6h"
#####
# list top differentially expressed genes
R.IAV.vs.Mock.nhbe = topTable(fit2, coef="TreatIAV", number=nrow(dge$counts))
dim(R.IAV.vs.Mock.nhbe[R.IAV.vs.Mock.nhbe$adj.P.Val < 0.05,])

## [1] 3163     6
head(R.IAV.vs.Mock.nhbe,10)

##          logFC    AveExpr         t    P.Value adj.P.Val       B
## HERPUD1  0.9919174  6.947284  12.109630 4.872615e-09 6.530766e-05 11.129857
## TNFAIP3  1.4427087  6.844549  11.332396 1.181067e-08 6.908801e-05 10.271476
## RPL39   -1.1693866  7.597367 -11.101131 1.551751e-08 6.908801e-05 10.026587
## SEL1L    1.5630318  6.658168  10.864357 2.061867e-08 6.908801e-05 9.743186
## HSP90B1  0.8207992  10.709018 10.326801 4.004951e-08 9.409930e-05 9.112786
## ZNFX1    1.2753692  7.335524  10.286767 4.212458e-08 9.409930e-05 9.055925
## MIR22HG  1.0089028  6.564312  10.120632 5.203375e-08 9.962976e-05 8.851143
## TFRC     1.8876586  7.315107  9.905429 6.868506e-08 1.119117e-04 8.582292
## JUND    -1.6427335  5.523348 -9.472161 1.218256e-07 1.632829e-04 8.008954
## ZNF106   0.9148682  5.841571  9.286129 1.567446e-07 1.750706e-04 7.771335

R.IFNB12h.vs.Mock.nhbe = topTable(fit2, coef="TreatIFNB12h", number=nrow(dge$counts))
dim(R.IFNB12h.vs.Mock.nhbe[R.IFNB12h.vs.Mock.nhbe$adj.P.Val < 0.05,])

```

```

## [1] 2337      6
head(R.IFN12h.vs.Mock.nhbe,10)

##          logFC    AveExpr      t     P.Value   adj.P.Val      B
## SP100  2.582294 7.135342 20.45535 3.369869e-12 2.131779e-08 18.30870
## LAP3   2.527329 6.260238 19.83136 5.232015e-12 2.131779e-08 17.85732
## HLA-B  3.705772 5.956073 19.55971 6.362095e-12 2.131779e-08 17.63500
## SAMHD1 4.516819 5.334821 19.64465 5.983121e-12 2.131779e-08 17.54236
## TRIM21  2.664225 6.171243 18.85319 1.070887e-11 2.870619e-08 17.16025
## ISG20   2.809192 5.241881 18.17396 1.797515e-11 3.352045e-08 16.63798
## MYD88   1.980672 7.267599 18.03631 2.000773e-11 3.352045e-08 16.60863
## SP140L  2.060598 4.880210 18.04198 1.991930e-11 3.352045e-08 16.51238
## CASP7   1.753695 5.981656 16.09754 9.850439e-11 1.143927e-07 15.03867
## TRIM25  2.772920 7.350617 15.82980 1.244302e-10 1.143927e-07 14.81767

R.IFN4h.vs.Mock.nhbe = topTable(fit2, coef="TreatIFNB4h", number=nrow(dge$counts))
dim(R.IFN4h.vs.Mock.nhbe[R.IFN4h.vs.Mock.nhbe$adj.P.Val < 0.05,])

## [1] 2197      6
head(R.IFN4h.vs.Mock.nhbe,10)

##          logFC    AveExpr      t     P.Value   adj.P.Val      B
## TRIM21  2.719421 6.171243 19.39795 7.156485e-12 9.591837e-08 17.35663
## IRF1    2.472172 4.758509 15.40326 1.818843e-10 5.300472e-07 14.26860
## ZNFX1   2.222788 7.335524 15.20347 2.179844e-10 5.300472e-07 14.23256
## SP100   1.909989 7.135342 15.11066 2.372815e-10 5.300472e-07 14.14874
## GBP1    4.890208 5.421724 15.39743 1.828428e-10 5.300472e-07 14.14379
## SAMHD1  3.444213 5.334821 14.91185 2.850099e-10 5.457126e-07 13.81930
## SLFN5   1.689615 8.155478 14.69731 3.481852e-10 5.833407e-07 13.78737
## CD274   2.408593 5.879768 14.17901 5.709082e-10 7.091418e-07 13.26906
## MYD88   1.544091 7.267599 14.08429 6.259684e-10 7.091418e-07 13.21005
## RGMB    1.648073 5.817383 14.09131 6.217008e-10 7.091418e-07 13.19490

R.IFN6h.vs.Mock.nhbe = topTable(fit2, coef="TreatIFNB6h", number=nrow(dge$counts))
dim(R.IFN6h.vs.Mock.nhbe[R.IFN6h.vs.Mock.nhbe$adj.P.Val < 0.05,])

## [1] 3992      6
head(R.IFN6h.vs.Mock.nhbe,10)

##          logFC    AveExpr      t     P.Value   adj.P.Val      B
## TRIM21  2.839614 6.171243 20.15748 4.150741e-12 5.563238e-08 17.99047
## SLFN5   2.134432 8.155478 18.56229 1.333966e-11 7.626148e-08 16.98416
## SAMHD1  4.172719 5.334821 18.10362 1.898483e-11 7.626148e-08 16.42030
## IRF1    2.872830 4.758509 17.87200 2.275953e-11 7.626148e-08 16.29557
## SP100   2.220380 7.135342 17.52534 2.997796e-11 8.035891e-08 16.18836
## ZNFX1   2.442077 7.335524 16.65295 6.133710e-11 1.306037e-07 15.49876
## MYD88   1.806991 7.267599 16.43376 7.381795e-11 1.306037e-07 15.32124
## RGMB    1.898915 5.817383 16.18644 9.122197e-11 1.358498e-07 15.08794
## GBP1    5.206216 5.421724 16.36973 7.795492e-11 1.306037e-07 15.05752
## TRIM25  2.807578 7.350617 16.04780 1.028466e-10 1.378453e-07 14.99519

```

Series 16: A549.ACE2_SARS.CoV.2 vs A549.ACE2_Mock (MOI=2)

```

new.data <- count.table[,c(43:48)]
Series <- colnames(count.table)[c(43:48)]

```

```

Treat <- c(rep("Mock",3),rep("SARS",3))
new.meta <- data.frame(Series = Series, Treat = Treat)
new.meta$Treat <- as.factor(new.meta$Treat)
new.meta$Treat <- relevel(new.meta$Treat, "Mock")

##### design matrix
new.design <- model.matrix(~ Treat, data = new.meta)
new.design

##   (Intercept) TreatSARS
## 1           1        0
## 2           1        0
## 3           1        0
## 4           1        1
## 5           1        1
## 6           1        1
## attr(,"assign")
## [1] 0 1
## attr(,"contrasts")
## attr(,"contrasts")$Treat
## [1] "contr.treatment"

##### filtering
# non-specific filtering
dge <- DGEList(counts = new.data)
counts.filt <- rowSums(cpm(dge) > 1) >=3
# Check what fraction of genes remain
sum(counts.filt)/nrow(dge)

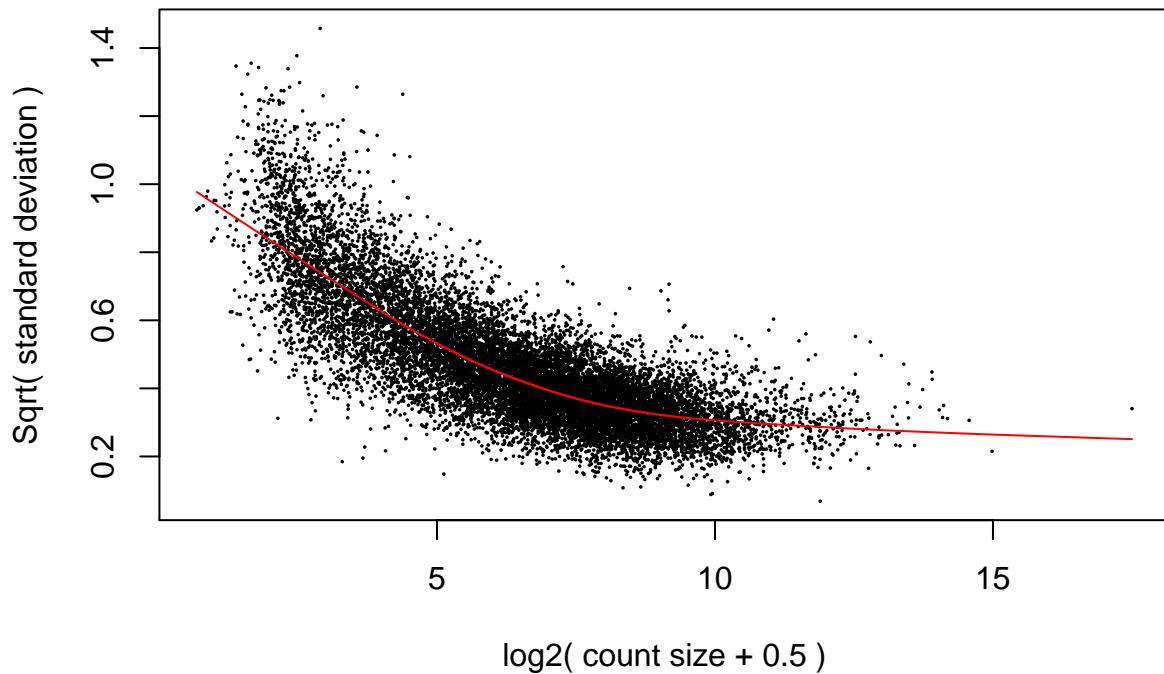
## [1] 0.4909252
# Remove low expressed genes.
dge <- dge[counts.filt,,keep.lib.sizes=T]

##### TMM normalization
dge <- calcNormFactors(dge, method="TMM")

#### VOOM
v.1 <- voom(dge,new.design,plot=TRUE)

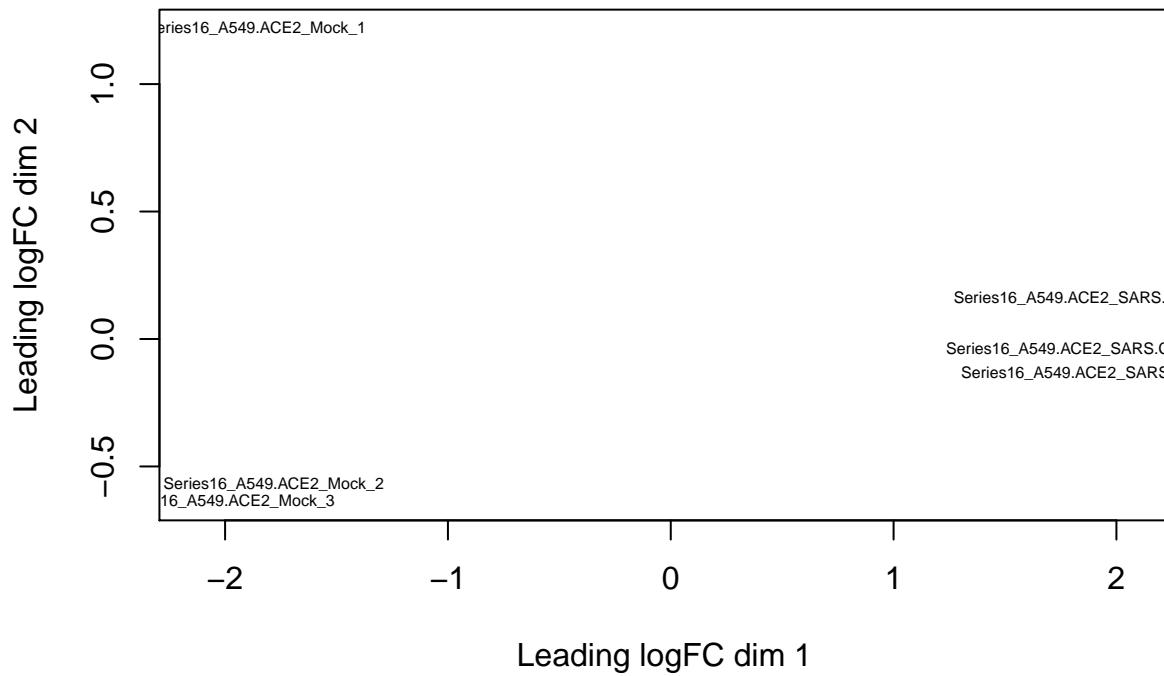
```

voom: Mean–variance trend



```
##### MDS plot  
plotMDS(v.1, main="plotMDS(v)", cex=0.5)
```

plotMDS(v)



```
##### fit linear model  
# finding differential expression by fitting linear models
```

```

fit <- lmFit(v.1,new.design)
# calculating the statistics
fit2 <- eBayes(fit)
colnames(fit2)

## [1] "(Intercept)" "TreatSARS"

##### list top differentially expressed genes
R.SARS.vs.Mock.A549ACE2.2 = topTable(fit2, coef="TreatSARS", number=nrow(dge$counts))
dim(R.SARS.vs.Mock.A549ACE2.2[R.SARS.vs.Mock.A549ACE2.2$adj.P.Val < 0.05,])

## [1] 9638     6
head(R.SARS.vs.Mock.A549ACE2.2,10)

##          logFC   AveExpr      t    P.Value adj.P.Val      B
## NFKBIA  5.209996 8.946323 70.93937 2.536966e-27 3.520295e-23 52.17928
## TNFAIP3  5.145118 8.446757 63.49455 2.826727e-26 1.777938e-22 49.89701
## CXCL8   4.839643 9.468618 62.27390 4.309977e-26 1.777938e-22 49.69705
## PPP1R15A 4.844438 9.074437 61.77932 5.125218e-26 1.777938e-22 49.48499
## TIPARP   3.707104 8.463826 52.46985 1.775212e-24 4.926569e-21 46.10993
## CXCL2   5.273084 7.063022 51.99796 2.159265e-24 4.993661e-21 45.51405
## EFNA1    4.643756 7.669249 51.27790 2.921253e-24 5.790757e-21 45.48626
## CHD2     3.652059 8.725519 49.32770 6.767088e-24 1.173751e-20 44.84447
## CCNL1    3.795424 8.176138 47.35995 1.633731e-23 2.518850e-20 43.95256
## CSRNPI   4.064361 7.316676 44.79029 5.460704e-23 7.577272e-20 42.69292

## save results in Excel format.
library(WriteXLS)
# make list of data.frames to write to xls file
sigGenes = list()
sigGenes[["NHBE_SARS.CoV.2_vs_NHBE_Mock"]] = R.SARS.vs.Mock.nhbe
sigGenes[["NHBE_IAV_vs_NHBE_Mock"]] = R.IAV.vs.Mock.nhbe
sigGenes[["NHBE_IFNB12h_vs_NHBE_Mock"]] = R.IFNB12h.vs.Mock.nhbe
sigGenes[["NHBE_IFNB6h_vs_NHBE_Mock"]] = R.IFNB6h.vs.Mock.nhbe
sigGenes[["NHBE_IFNB4h_vs_NHBE_Mock"]] = R.IFNB4h.vs.Mock.nhbe

sigGenes[["A549_SARS.CoV.2_vs_A549_Mock_S2"]] = R.SARS.vs.Mock.A549
sigGenes[["A549_SARS.CoV.2_vs_A549_Mock_S5"]] = R.SARS.vs.Mock.A549.2
sigGenes[["A549_IAV_vs_A549_Mock"]] = R.IAV.vs.Mock.A549

sigGenes[["Calu3_SARS.CoV.2_vs_Calu3_Mock"]] = R.SARS.vs.Mock.Calu3
sigGenes[["A549.ACE2_SARS_vs_Mock_S6"]] = R.SARS.vs.Mock.A549ACE2
sigGenes[["A549.ACE2_SARS_vs_Mock_S16"]] = R.SARS.vs.Mock.A549ACE2.2

sigGenes_fdr05 = lapply(sigGenes, function(x) x[x[, "adj.P.Val"] <= 0.05,]) # limit to FDR of 0.05

# print number of significant genes
lapply(sigGenes_fdr05, nrow)

## $NHBE_SARS.CoV.2_vs_NHBE_Mock
## [1] 390
##
## $NHBE_IAV_vs_NHBE_Mock
## [1] 3163
##

```

```

## $NHBE_IFNB12h_vs_NHBE_Mock
## [1] 2337
##
## $NHBE_IFNB6h_vs_NHBE_Mock
## [1] 3992
##
## $NHBE_IFNB4h_vs_NHBE_Mock
## [1] 2197
##
## $A549_SARS.CoV.2_vs_A549_Mock_S2
## [1] 154
##
## $A549_SARS.CoV.2_vs_A549_Mock_S5
## [1] 9046
##
## $A549_IAV_vs_A549_Mock
## [1] 0
##
## $Calu3_SARS.CoV.2_vs_Calu3_Mock
## [1] 7337
##
## $A549.ACE2_SARS_vs_Mock_S6
## [1] 5364
##
## $A549.ACE2_SARS_vs_Mock_S16
## [1] 9638

# write out results in Excel format
WriteXLS("sigGenes_fdr05",ExcelFileName=paste("DEGList_1.xls", sep=""),
         row.names=T, FreezeRow=1,FreezeCol=1,AdjWidth=F)

library(EnhancedVolcano)

## Loading required package: ggrepel

## Registered S3 methods overwritten by 'ggalt':
##   method           from
##   grid.draw.absoluteGrob  ggplot2
##   grobHeight.absoluteGrob ggplot2
##   grobWidth.absoluteGrob ggplot2
##   grobX.absoluteGrob    ggplot2
##   grobY.absoluteGrob    ggplot2

EnhancedVolcano(R.SARS.vs.Mock.nhbe,
                 lab = rownames(R.SARS.vs.Mock.nhbe),
                 x = 'logFC',
                 y = 'adj.P.Val',
                 pCutoff = 1e-3,
                 selectLab = unique(c("IL6", "CXCL5", "CXCL2", "CXCL3",
                                      "CXCL8", "CXCL1", "IL1B", "IL32", "CCL20",
                                      row.names(R.SARS.vs.Mock.nhbe[1:20,]))),
                 labSize = 2, subtitle = "NHBE_SARS.CoV.2 vs NHBE_Mock", titleLabSize = 12,
                 subtitleLabSize = 10,
                 title = "A") -> vp1
EnhancedVolcano(R.IAV.vs.Mock.nhbe,
                 lab = rownames(R.IAV.vs.Mock.nhbe),

```

```

x = 'logFC',
y = 'adj.P.Val',
#selectLab = unique(c("IL6", "CXCL5", "CXCL2", "CXCL3",
#                      "CXCL8", "CXCL1", "IL1B", "IL32", "CCL20"
#                      )),
pCutoff = 1e-3,labSize =2,
subtitle = "NHBE_IAV vs NHBE_Mock",titleLabSize = 12,
subtitleLabSize = 10,
title = "B") -> vp2

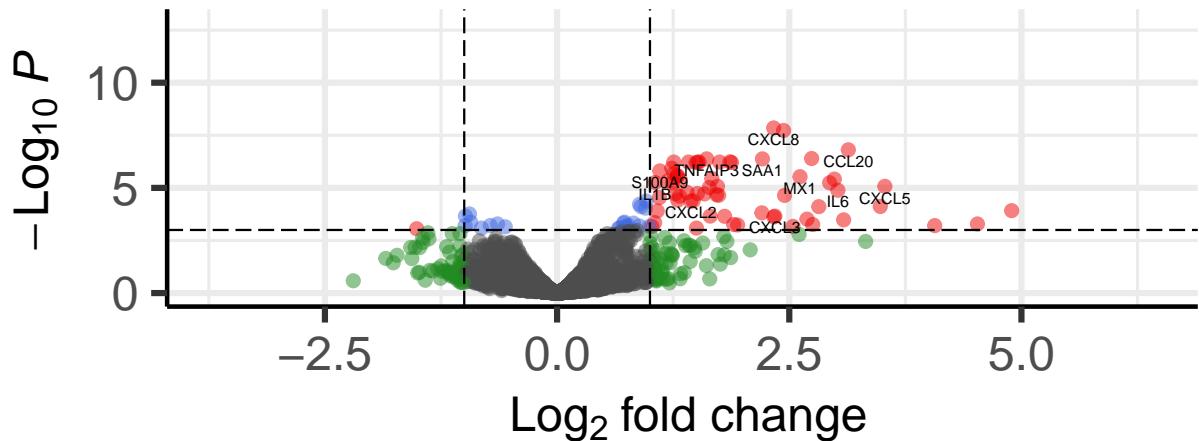
```

vp1

A

NHBE_SARS-CoV.2 vs NHBE_Mock

● NS ● Log₂ FC ● p-value ● p-value and log₂ FC

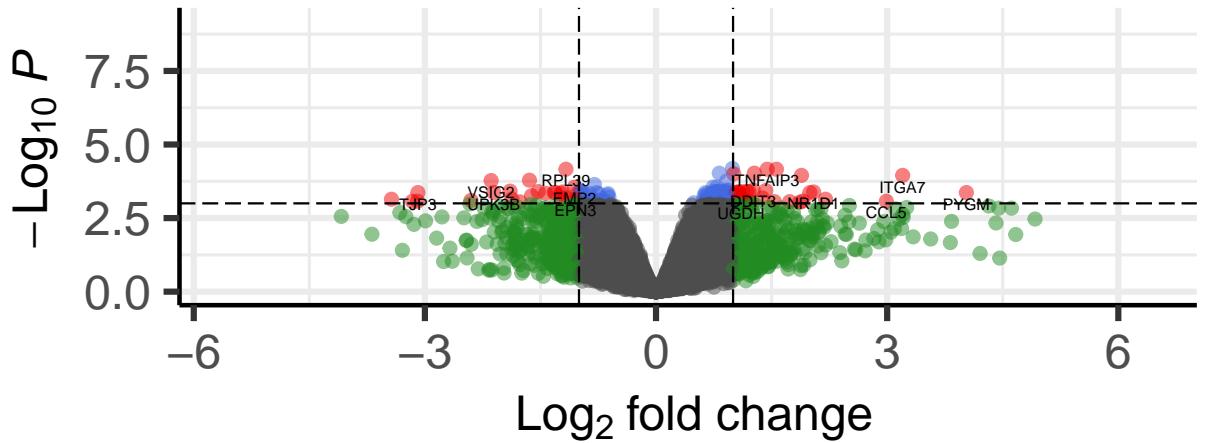


vp2

B

NHBE_IAV vs NHBE_Mock

● NS ● Log₂ FC ● p-value ● p-value and log₂ FC



total = 13403 variables

GO enrichment analysis

```

library(BiasedUrn)
library(org.Hs.eg.db)

## Loading required package: AnnotationDbi
## Loading required package: stats4
## Loading required package: BiocGenerics
## Loading required package: parallel
##
## Attaching package: 'BiocGenerics'
## 
## The following objects are masked from 'package:parallel':
## 
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB

## The following object is masked from 'package:limma':
## 
##     plotMA

## The following objects are masked from 'package:dplyr':
## 
##     combine, intersect, setdiff, union

## The following objects are masked from 'package:stats':
## 
##     
```

```

##      IQR, mad, sd, var, xtabs
## The following objects are masked from 'package:base':
##
##      anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##      dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##      grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##      order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##      rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##      union, unique, unsplit, which.max, which.min

## Loading required package: Biobase

## Welcome to Bioconductor
##
##      Vignettes contain introductory material; view with
##      'browseVignettes()'. To cite Bioconductor, see
##      'citation("Biobase")', and for packages 'citation("pkgname")'.

## Loading required package: IRanges

## Loading required package: S4Vectors

##
## Attaching package: 'S4Vectors'

## The following object is masked from 'package:tidyr':
##
##      expand

## The following objects are masked from 'package:dplyr':
##
##      first, rename

## The following object is masked from 'package:base':
##
##      expand.grid

##
## Attaching package: 'IRanges'

## The following object is masked from 'package:purrr':
##
##      reduce

## The following objects are masked from 'package:dplyr':
##
##      collapse, desc, slice

##
## Attaching package: 'AnnotationDbi'

## The following object is masked from 'package:dplyr':
##
##      select

##
library(GO.db)

##

```

```

library(clusterProfiler)

## clusterProfiler v3.18.1 For help: https://guangchuangyu.github.io/software/clusterProfiler
##
## If you use clusterProfiler in published research, please cite:
## Guangchuang Yu, Li-Gen Wang, Yanyan Han, Qing-Yu He. clusterProfiler: an R package for comparing bio
##
## Attaching package: 'clusterProfiler'

## The following object is masked from 'package:AnnotationDbi':
##
##      select

## The following object is masked from 'package:IRanges':
##
##      slice

## The following object is masked from 'package:S4Vectors':
##
##      rename

## The following object is masked from 'package:purrr':
##
##      simplify

## The following object is masked from 'package:stats':
##
##      filter

library(stringr)

```

GO enrichment for SARS-CoV-2_vs_Mock in NHBE

```

updiff <- subset(R.SARS.vs.Mock.nhbe, logFC > 0 & adj.P.Val < 0.05)
upgene <- rownames(updiff)
gene_name=as.vector(upgene)
geneID <- mget(gene_name, org.Hs.egSYMBOL2EG, ifnotfound=NA)%>%as.character()
data=cbind(Gene=upgene,entrezID=geneID)%>%as.data.frame()
go.SARS.vs.Mock.nhbe <- enrichGO(gene=data$entrezID, OrgDb = org.Hs.eg.db, ont='BP',pvalueCutoff = 0.05)
# go.SARS.vs.Mock.nhbe <- subset(R.SARS.vs.Mock.nhbe, logFC > 0 & adj.P.Val < 0.05)
goUP.SARS.vs.Mock.nhbe <- go.SARS.vs.Mock.nhbe[order(go.SARS.vs.Mock.nhbe$Count,decreasing = T)[1:10],]
goUP.SARS.vs.Mock.nhbe

##           ID                  Description
## GO:0009615 GO:0009615      response to virus
## GO:0032496 GO:0032496      response to lipopolysaccharide
## GO:0002237 GO:0002237      response to molecule of bacterial origin
## GO:0051607 GO:0051607      defense response to virus
## GO:0052548 GO:0052548      regulation of endopeptidase activity
## GO:0052547 GO:0052547      regulation of peptidase activity
## GO:0043312 GO:0043312      neutrophil degranulation
## GO:0002283 GO:0002283 neutrophil activation involved in immune response
## GO:0050727 GO:0050727      regulation of inflammatory response
## GO:0008544 GO:0008544      epidermis development
##          GeneRatio    BgRatio      pvalue     p.adjust      qvalue
## GO:0009615      36/241  349/18866  2.232505e-22 8.400916e-19 6.016013e-19

```

```

## GO:0032496 31/241 334/18866 4.907318e-18 6.155413e-15 4.407977e-15
## GO:0002237 31/241 356/18866 3.050111e-17 2.295514e-14 1.643849e-14
## GO:0051607 28/241 258/18866 3.608203e-18 6.155413e-15 4.407977e-15
## GO:0052548 28/241 434/18866 2.025252e-12 5.080683e-10 3.638348e-10
## GO:0052547 28/241 466/18866 1.106049e-11 2.190558e-09 1.568690e-09
## GO:0043312 27/241 487/18866 1.626462e-10 2.110474e-08 1.511340e-08
## GO:0002283 27/241 490/18866 1.864946e-10 2.339264e-08 1.675179e-08
## GO:0050727 26/241 425/18866 4.269644e-11 6.694446e-09 4.793986e-09
## GO:0008544 26/241 477/18866 5.227929e-10 5.604673e-08 4.013585e-08
##
## GO:0009615 7128/10379/4599/80149/3553/3429/330/4939/3569/4938/4940/8519/2537/10964/10561/4600/5359/23576/7128/56300/6279/2919/3383/6280/80149/3553/6374/3569/7128/10379/4599/80149/3553/3429/330/4939/3569/49279/718/6280/330/12/4318/2537/1992/6279/718/6280/330/12/4318/2537/1992/6279/2919/718/6280/12/4318/1999/6279/2919/718/6280/12/4318/1999/6288/7128/6279/10318/718/6280/803854/3624/6703/6700/23764/6704/5266/
##          Count
## GO:0009615    36
## GO:0032496    31
## GO:0002237    31
## GO:0051607    28
## GO:0052548    28
## GO:0052547    28
## GO:0043312    27
## GO:0002283    27
## GO:0050727    26
## GO:0008544    26
##
intf.SARS <- go.SARS.vs.Mock.nhbe %>%
  select(ID,Description,GeneRatio,BgRatio,pvalue,p.adjust,qvalue,geneID,Count) %>%
  filter(str_detect>Description,"type I interferon"))
intf.SARS

##           ID                               Description
## GO:0060337 GO:0060337 type I interferon signaling pathway
## GO:0071357 GO:0071357 cellular response to type I interferon
## GO:0034340 GO:0034340 response to type I interferon
## GO:0032479 GO:0032479 regulation of type I interferon production
## GO:0032606 GO:0032606 type I interferon production
## GO:0032480 GO:0032480 negative regulation of type I interferon production
## GO:0032481 GO:0032481 positive regulation of type I interferon production
##           GeneRatio   BgRatio      pvalue     p.adjust      qvalue
## GO:0060337    18/241 95/18866 2.011825e-16 1.081500e-13 7.744770e-14
## GO:0071357    18/241 95/18866 2.011825e-16 1.081500e-13 7.744770e-14
## GO:0034340    18/241 99/18866 4.351002e-16 2.046602e-13 1.465601e-13
## GO:0032479    11/241 125/18866 6.066865e-07 2.481480e-05 1.777022e-05
## GO:0032606    11/241 127/18866 7.116374e-07 2.818833e-05 2.018606e-05
## GO:0032480     5/241 47/18866 3.231780e-04 4.026884e-03 2.883708e-03
## GO:0032481     6/241 74/18866 3.662812e-04 4.446181e-03 3.183972e-03
##
## GO:0060337 10379/4599/3429/4939/4938/4940/54739/8519/2537/4600/25939/10410/6772/684/3665/9641/3434/3434/gen

```

```

## GO:0071357 10379/4599/3429/4939/4938/4940/54739/8519/2537/4600/25939/10410/6772/684/3665/9641/3434/3
## GO:0034340 10379/4599/3429/4939/4938/4940/54739/8519/2537/4600/25939/10410/6772/684/3665/9641/3434/3
## GO:0032479                                         7128/4791/4790/6772/64135/3665/9641/7187/7097/5971/9
## GO:0032606                                         7128/4791/4790/6772/64135/3665/9641/7187/7097/5971/9
## GO:0032480                                         7128/64135/9641/5971/9
## GO:0032481                                         4791/4790/6772/64135/3665/7
##             Count
## GO:0060337     18
## GO:0071357     18
## GO:0034340     18
## GO:0032479     11
## GO:0032606     11
## GO:0032480      5
## GO:0032481      6

chemok.SARS <- go.SARS.vs.Mock.nhbe %>%
  select(ID,Description,GeneRatio,BgRatio,pvalue,p.adjust,qvalue,geneID,Count) %>%
  filter(str_detect>Description,"chemokine"))
chemok.SARS

##           ID                  Description GeneRatio   BgRatio
## GO:1990868 GO:1990868      response to chemokine 10/241 97/18866
## GO:1990869 GO:1990869      cellular response to chemokine 10/241 97/18866
## GO:0070098 GO:0070098 chemokine-mediated signaling pathway 8/241 88/18866
## GO:0032602 GO:0032602      chemokine production 7/241 75/18866
## GO:0032642 GO:0032642 regulation of chemokine production 5/241 68/18866
##           pvalue    p.adjust    qvalue
## GO:1990868 4.566132e-07 0.0000195254 1.398241e-05
## GO:1990869 4.566132e-07 0.0000195254 1.398241e-05
## GO:0070098 1.683542e-05 0.0003519538 2.520390e-04
## GO:0032602 4.824719e-05 0.0008290145 5.936689e-04
## GO:0032642 1.767860e-03 0.0148492360 1.063375e-02
##           geneID Count
## GO:1990868 3576/6364/2919/80149/6374/2920/2921/1906/6372/1843    10
## GO:1990869 3576/6364/2919/80149/6374/2920/2921/1906/6372/1843    10
## GO:0070098            3576/6364/2919/6374/2920/2921/1906/6372      8
## GO:0032602            6279/6280/3553/3569/6372/7097/1969      7
## GO:0032642            3553/3569/6372/7097/1969      5

cytokine.SARS <- go.SARS.vs.Mock.nhbe %>%
  select(ID,Description,GeneRatio,BgRatio,pvalue,p.adjust,qvalue,geneID,Count) %>%
  filter(str_detect>Description,"cytokine"))
cytokine.SARS

##           ID                  Description GeneRatio
## GO:0001819 GO:0001819 positive regulation of cytokine production 23/241
## GO:0060759 GO:0060759 regulation of response to cytokine stimulus 14/241
## GO:0001959 GO:0001959 regulation of cytokine-mediated signaling pathway 12/241
## GO:0060760 GO:0060760 positive regulation of response to cytokine stimulus 6/241

```

```

## GO:0001818           negative regulation of cytokine production      14/241
## GO:0001961 positive regulation of cytokine-mediated signaling pathway 4/241
##          BgRatio      pvalue      p.adjust      qvalue
## GO:0001819 447/18866 1.621097e-08 1.220038e-06 8.736862e-07
## GO:0060759 196/18866 2.374445e-07 1.175663e-05 8.419086e-06
## GO:0001959 183/18866 4.267772e-06 1.244932e-04 8.915134e-05
## GO:0060760 57/18866 8.593801e-05 1.370274e-03 9.812726e-04
## GO:0001818 360/18866 2.318611e-04 3.127217e-03 2.239444e-03
## GO:0001961 50/18866 3.774721e-03 2.710685e-02 1.941159e-02
##
## GO:0001819 6288/718/3553/330/3569/4791/3552/1437/101/4790/6772/64135/115362/3665/602/5452/2152/5724/
## GO:0060759                                         7128/330/3569/3656/1906/25939/9021/83666/6772/64
## GO:0001959                                         7128/330/3569/3656/1906/25939/9021/83
## GO:0060760                                         1906/836
## GO:0001818                                         7128/3624/80149/1992/4790/684/64135/602/9641/5
## GO:0001961
##          Count
## GO:0001819    23
## GO:0060759    14
## GO:0001959    12
## GO:0060760     6
## GO:0001818    14
## GO:0001961     4

inflammatory.SARS <- go.SARS.vs.Mock.nhbe %>%
  select(ID,Description,GeneRatio,BgRatio,pvalue,p.adjust,qvalue,geneID,Count) %>%
  filter(str_detect>Description,"inflammatory"))
inflammatory.SARS

```

	ID	Description				
##						
## GO:0002526	GO:0002526	acute inflammatory response				
## GO:0050727	GO:0050727	regulation of inflammatory response				
## GO:0050729	GO:0050729	positive regulation of inflammatory response				
## GO:0150076	GO:0150076	neuroinflammatory response				
## GO:0002675	GO:0002675	positive regulation of acute inflammatory response				
## GO:0002544	GO:0002544	chronic inflammatory response				
## GO:0150077	GO:0150077	regulation of neuroinflammatory response				
## GO:0002673	GO:0002673	regulation of acute inflammatory response				
## GO:0002269	GO:0002269	leukocyte activation involved in inflammatory response				
## GO:0002523	GO:0002523	leukocyte migration involved in inflammatory response				
## GO:0006925	GO:0006925	inflammatory cell apoptotic process				
## GO:0050728	GO:0050728	negative regulation of inflammatory response				
##	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	
## GO:0002526	16/241	111/18866	8.478547e-13	2.604727e-10	1.865281e-10	
## GO:0050727	26/241	425/18866	4.269644e-11	6.694446e-09	4.793986e-09	
## GO:0050729	16/241	158/18866	2.007664e-10	2.437045e-08	1.745202e-08	
## GO:0150076	8/241	77/18866	6.217485e-06	1.683194e-04	1.205359e-04	
## GO:0002675	5/241	31/18866	4.227024e-05	7.432846e-04	5.322765e-04	
## GO:0002544	4/241	20/18866	1.071315e-04	1.632129e-03	1.168790e-03	
## GO:0150077	5/241	43/18866	2.113941e-04	2.892639e-03	2.071459e-03	
## GO:0002673	5/241	49/18866	3.935128e-04	4.730954e-03	3.387902e-03	
## GO:0002269	5/241	50/18866	4.327317e-04	5.088654e-03	3.644056e-03	
## GO:0002523	3/241	16/18866	1.019430e-03	9.947268e-03	7.123378e-03	
## GO:0006925	3/241	22/18866	2.649402e-03	2.068402e-02	1.481212e-02	
## GO:0050728	8/241	196/18866	3.781848e-03	2.710685e-02	1.941159e-02	

```

##                                     6289/6288/6279/3383/718/3553/3569,
## GO:0002526
## GO:0050727 6288/7128/6279/10318/718/6280/80149/3553/330/3569/4792/64332/4318/9021/7052/101/4790/1153
## GO:0050729                                     6279/10318/718/6280/3553/3569/4792/64332,
## GO:0150076
## GO:0002675
## GO:0002544
## GO:0150077
## GO:0002673
## GO:0002269
## GO:0002523
## GO:0006925
## GO:0050728
##          Count
## GO:0002526    16
## GO:0050727    26
## GO:0050729    16
## GO:0150076     8
## GO:0002675     5
## GO:0002544     4
## GO:0150077     5
## GO:0002673     5
## GO:0002269     5
## GO:0002523     3
## GO:0006925     3
## GO:0050728     8

SARS.dif <- rbind(intf.SARS,chemok.SARS,cytokine.SARS,inflammatory.SARS)
SARS.dif <- subset(SARS.dif, p.adjust<0.001 )
SARS.dif

##          ID                                     Description
## GO:0060337 GO:0060337      type I interferon signaling pathway
## GO:0071357 GO:0071357      cellular response to type I interferon
## GO:0034340 GO:0034340      response to type I interferon
## GO:0032479 GO:0032479      regulation of type I interferon production
## GO:0032606 GO:0032606      type I interferon production
## GO:1990868 GO:1990868      response to chemokine
## GO:1990869 GO:1990869      cellular response to chemokine
## GO:0070098 GO:0070098      chemokine-mediated signaling pathway
## GO:0032602 GO:0032602      chemokine production
## GO:0001819 GO:0001819      positive regulation of cytokine production
## GO:0060759 GO:0060759      regulation of response to cytokine stimulus
## GO:0001959 GO:0001959      regulation of cytokine-mediated signaling pathway
## GO:0002526 GO:0002526      acute inflammatory response
## GO:0050727 GO:0050727      regulation of inflammatory response
## GO:0050729 GO:0050729      positive regulation of inflammatory response
## GO:0150076 GO:0150076      neuroinflammatory response
## GO:0002675 GO:0002675      positive regulation of acute inflammatory response
##          GeneRatio   BgRatio      pvalue      p.adjust      qvalue
## GO:0060337    18/241  95/18866  2.011825e-16  1.081500e-13  7.744770e-14
## GO:0071357    18/241  95/18866  2.011825e-16  1.081500e-13  7.744770e-14
## GO:0034340    18/241  99/18866  4.351002e-16  2.046602e-13  1.465601e-13
## GO:0032479    11/241  125/18866 6.066865e-07  2.481480e-05  1.777022e-05
## GO:0032606    11/241  127/18866 7.116374e-07  2.818833e-05  2.018606e-05

```

```

## GO:1990868 10/241 97/18866 4.566132e-07 1.952540e-05 1.398241e-05
## GO:1990869 10/241 97/18866 4.566132e-07 1.952540e-05 1.398241e-05
## GO:0070098 8/241 88/18866 1.683542e-05 3.519538e-04 2.520390e-04
## GO:0032602 7/241 75/18866 4.824719e-05 8.290145e-04 5.936689e-04
## GO:0001819 23/241 447/18866 1.621097e-08 1.220038e-06 8.736862e-07
## GO:0060759 14/241 196/18866 2.374445e-07 1.175663e-05 8.419086e-06
## GO:0001959 12/241 183/18866 4.267772e-06 1.244932e-04 8.915134e-05
## GO:0002526 16/241 111/18866 8.478547e-13 2.604727e-10 1.865281e-10
## GO:0050727 26/241 425/18866 4.269644e-11 6.694446e-09 4.793986e-09
## GO:0050729 16/241 158/18866 2.007664e-10 2.437045e-08 1.745202e-08
## GO:0150076 8/241 77/18866 6.217485e-06 1.683194e-04 1.205359e-04
## GO:0002675 5/241 31/18866 4.227024e-05 7.432846e-04 5.322765e-04
##
## GO:0060337 10379/4599/3429/4939/4938/4940/54739/8519/2537/4600
## GO:0071357 10379/4599/3429/4939/4938/4940/54739/8519/2537/4600
## GO:0034340 10379/4599/3429/4939/4938/4940/54739/8519/2537/4600
## GO:0032479 7128/4791/4790
## GO:0032606 7128/4791/4790
## GO:1990868 3576/6364
## GO:1990869 3576/6364
## GO:0070098
## GO:0032602
## GO:0001819 6288/718/3553/330/3569/4791/3552/1437/101/4790/6772/64135/115362/3665/6
## GO:0060759 7128/330/3569/3656/1906/25939/
## GO:0001959 7128/330/3569/3656/
## GO:0002526 6289/6288/6279/3383/718/3553/3569
## GO:0050727 6288/7128/6279/10318/718/6280/80149/3553/330/3569/4792/64332/4318/9021/7052/101/4790/1153
## GO:0050729 6279/10318/718/6280/3553/3569/4792/64332
## GO:0150076
## GO:0002675
## Count
## GO:0060337 18
## GO:0071357 18
## GO:0034340 18
## GO:0032479 11
## GO:0032606 11
## GO:1990868 10
## GO:1990869 10
## GO:0070098 8
## GO:0032602 7
## GO:0001819 23
## GO:0060759 14
## GO:0001959 12
## GO:0002526 16
## GO:0050727 26
## GO:0050729 16
## GO:0150076 8
## GO:0002675 5

```

GO enrichment for IFNB_vs_Mock in NHBE

```

updiff <- subset(R.IFN4h.vs.Mock.nhbe, logFC > 0 & adj.P.Val < 0.05)
upgene <- rownames(updiff)
gene_name=as.vector(upgene)

```

```

geneID <- mget(gene_name, org.Hs.egSYMBOL2EG, ifnotfound=NA)%>%as.character()
data=cbind(Gene=upgene,entrezID=geneID)%>%as.data.frame()
go.IFN4h.vs.Mock.nhbe <- enrichGO(gene=data$entrezID, OrgDb = org.Hs.eg.db, ont='BP',pvalueCutoff = 0.05)
goUP.IFN4h.vs.Mock.nhbe <- go.IFN4h.vs.Mock.nhbe[order(go.IFN4h.vs.Mock.nhbe$p.adjust)[1:10],]
goUP.IFN4h.vs.Mock.nhbe

##          ID                Description GeneRatio
## GO:0051607 GO:0051607 defense response to virus 77/1188
## GO:0009615 GO:0009615             response to virus 88/1188
## GO:0034340 GO:0034340           response to type I interferon 42/1188
## GO:0060337 GO:0060337   type I interferon signaling pathway 40/1188
## GO:0071357 GO:0071357 cellular response to type I interferon 40/1188
## GO:0019058 GO:0019058            viral life cycle 77/1188
## GO:0034341 GO:0034341       response to interferon-gamma 55/1188
## GO:1903900 GO:1903900 regulation of viral life cycle 47/1188
## GO:0048525 GO:0048525 negative regulation of viral process 37/1188
## GO:0045069 GO:0045069 regulation of viral genome replication 36/1188
##          BgRatio      pvalue     p.adjust    qvalue
## GO:0051607 258/18866 6.222405e-32 3.523748e-28 2.587865e-28
## GO:0009615 349/18866 2.843256e-30 8.050680e-27 5.912476e-27
## GO:0034340 99/18866 9.149202e-25 1.727064e-21 1.268368e-21
## GO:0060337 95/18866 1.691582e-23 1.915886e-20 1.407040e-20
## GO:0071357 95/18866 1.691582e-23 1.915886e-20 1.407040e-20
## GO:0019058 341/18866 2.467653e-23 2.329053e-20 1.710473e-20
## GO:0034341 202/18866 4.991287e-21 4.037951e-18 2.965500e-18
## GO:1903900 157/18866 5.155060e-20 3.649138e-17 2.679953e-17
## GO:0048525 104/18866 7.305362e-19 4.596696e-16 3.375846e-16
## GO:0045069 100/18866 1.383405e-18 7.834224e-16 5.753510e-16
##
## GO:0051607                                         3659/2633/25939/5371/3665/4940/24138/56829/10475/151636/6773/3669/23586/8638/91543/55337/
## GO:0009615                                         3659/2633/25939/5371/3665/4940/24138/56829/10475/151636/6773/3669/23586/8638/91543/55337/
## GO:0034340
## GO:0060337
## GO:0071357
## GO:0019058                                         6737/5371/3383/7706/4940/
## GO:0034341
## GO:1903900
## GO:0048525
## GO:0045069

##          Count
## GO:0051607    77
## GO:0009615    88
## GO:0034340    42
## GO:0060337    40
## GO:0071357    40
## GO:0019058    77
## GO:0034341    55
## GO:1903900    47
## GO:0048525    37
## GO:0045069    36

updiff <- subset(R.IFN6h.vs.Mock.nhbe, logFC > 0 & adj.P.Val < 0.05)
upgene <- rownames(updiff)
gene_name=as.vector(upgene)

```

```

geneID <- mget(gene_name, org.Hs.egSYMBOL2EG, ifnotfound=NA)%>%as.character()
data=cbind(Gene=upgene,entrezID=geneID)%>%as.data.frame()
go.IFN6h.vs.Mock.nhbe <- enrichGO(gene=data$entrezID, OrgDb = org.Hs.eg.db, ont='BP',pvalueCutoff = 0.05)
goUP.IFN6h.vs.Mock.nhbe <- go.IFN6h.vs.Mock.nhbe[order(go.IFN6h.vs.Mock.nhbe$p.adjust)[1:10],]
goUP.IFN6h.vs.Mock.nhbe

## ID Description GeneRatio
## GO:0051607 defense response to virus 87/2021
## GO:0009615 response to virus 102/2021
## GO:0019058 viral life cycle 98/2021
## GO:0034340 response to type I interferon 47/2021
## GO:0060337 type I interferon signaling pathway 45/2021
## GO:0071357 cellular response to type I interferon 45/2021
## GO:0034341 response to interferon-gamma 66/2021
## GO:1903900 regulation of viral life cycle 56/2021
## GO:0048525 negative regulation of viral process 42/2021
## GO:0045069 regulation of viral genome replication 41/2021
## BgRatio pvalue p.adjust qvalue
## GO:0051607 258/18866 1.614654e-23 9.809025e-20 7.186062e-20
## GO:0009615 349/18866 5.908893e-22 1.794826e-18 1.314884e-18
## GO:0019058 341/18866 1.456373e-20 2.949156e-17 2.160543e-17
## GO:0034340 99/18866 2.505130e-20 3.804666e-17 2.787286e-17
## GO:0060337 95/18866 1.793613e-19 1.816033e-16 1.330420e-16
## GO:0071357 95/18866 1.793613e-19 1.816033e-16 1.330420e-16
## GO:0034341 202/18866 2.337845e-17 2.028916e-14 1.486377e-14
## GO:1903900 157/18866 7.333328e-17 5.568746e-14 4.079646e-14
## GO:0048525 104/18866 3.468564e-15 2.341281e-12 1.715215e-12
## GO:0045069 100/18866 3.946055e-15 2.397229e-12 1.756202e-12
##
## GO:0051607 25939/3659/2633/5371/10475/24138/7128/4940/3665/3669/151636/56829/5610/6773/2635/55337/911
## GO:0009615 25939/3659/2633/5371/10475/24138/7128/4940/3665/3669/151636/56829/5610/6773/2635/55337/911
## GO:0019058 6737/7706/5371/10475/24138/3383/4940/3669/56829/5610/55337/911
## GO:0034340
## GO:0060337
## GO:0071357
## GO:0034341
## GO:1903900
## GO:0048525
## GO:0045069
## Count
## GO:0051607 87
## GO:0009615 102
## GO:0019058 98
## GO:0034340 47
## GO:0060337 45
## GO:0071357 45
## GO:0034341 66
## GO:1903900 56
## GO:0048525 42
## GO:0045069 41

updiff <- subset(R.IFN6h.vs.Mock.nhbe, logFC > 0 & adj.P.Val < 0.05)
upgene <- rownames(updiff)
gene_name=as.vector(upgene)

```

```

geneID <- mget(gene_name, org.Hs.egSYMBOL2EG, ifnotfound=NA)%>%as.character()
data=cbind(Gene=upgene,entrezID=geneID)%>%as.data.frame()
go.IFNB12h.vs.Mock.nhbe <- enrichGO(gene=data$entrezID, OrgDb = org.Hs.eg.db, ont='BP',pvalueCutoff = 0
goUP.IFNB12h.vs.Mock.nhbe <- go.IFNB12h.vs.Mock.nhbe[order(go.IFNB12h.vs.Mock.nhbe$p.adjust)[1:10],]
goUP.IFNB12h.vs.Mock.nhbe

##                                     ID          Description GeneRatio
## GO:0051607 GO:0051607 defense response to virus 81/1283
## GO:0009615 GO:0009615             response to virus 89/1283
## GO:0034340 GO:0034340           response to type I interferon 46/1283
## GO:0060337 GO:0060337      type I interferon signaling pathway 44/1283
## GO:0071357 GO:0071357 cellular response to type I interferon 44/1283
## GO:0034341 GO:0034341           response to interferon-gamma 58/1283
## GO:0002831 GO:0002831 regulation of response to biotic stimulus 82/1283
## GO:0071346 GO:0071346 cellular response to interferon-gamma 49/1283
## GO:0019058 GO:0019058           viral life cycle 69/1283
## GO:1903900 GO:1903900 regulation of viral life cycle 44/1283
##          BgRatio      pvalue    p.adjust     qvalue
## GO:0051607 258/18866 6.306964e-33 3.589293e-29 2.774400e-29
## GO:0009615 349/18866 1.587772e-28 4.518006e-25 3.492263e-25
## GO:0034340 99/18866 1.080616e-27 2.049929e-24 1.584524e-24
## GO:0060337 95/18866 1.797364e-26 2.045760e-23 1.581302e-23
## GO:0071357 95/18866 1.797364e-26 2.045760e-23 1.581302e-23
## GO:0034341 202/18866 1.000678e-21 9.491433e-19 7.336551e-19
## GO:0002831 409/18866 3.660059e-19 2.975628e-16 2.300058e-16
## GO:0071346 182/18866 2.758456e-17 1.962297e-14 1.516788e-14
## GO:0019058 341/18866 1.577539e-16 9.975305e-14 7.710568e-14
## GO:1903900 157/18866 2.283449e-16 1.299511e-13 1.004477e-13
##
## GO:0051607                               25939/3669/2633/4940/3665/55337/5359/85363/5371/10
## GO:0009615 25939/3669/2633/4940/3665/55337/5359/85363/5371/5610/151636/91543/8638/9636/2635/24138/10
## GO:0034340
## GO:0060337
## GO:0071357
## GO:0034341
## GO:0002831                               3106/25939/11274/3665/54625/9111/5359/85363/15
## GO:0071346
## GO:0019058
## GO:1903900

##          Count
## GO:0051607    81
## GO:0009615    89
## GO:0034340    46
## GO:0060337    44
## GO:0071357    44
## GO:0034341    58
## GO:0002831    82
## GO:0071346    49
## GO:0019058    69
## GO:1903900    44

```

GO enrichment for SARS-CoV-2_vs_Mock in A549

```

updiff <- subset(R.SARS.vs.Mock.A549.2, logFC > 0 & adj.P.Val < 0.05)
upgene <- rownames(updiff)
gene_name=as.vector(upgene)
geneID <- mget(gene_name, org.Hs.egSYMBOL2EG, ifnotfound=NA)%>%as.character()
data=cbind(Gene=upgene,entrezID=GeneID)%>%as.data.frame()
go.SARS.vs.Mock.A549.2 <- enrichGO(gene=data$entrezID, OrgDb = org.Hs.eg.db, ont='BP',pvalueCutoff = 0.05, method='exact')
goUP.SARS.vs.Mock.A549.2 <- go.SARS.vs.Mock.A549.2[order(go.SARS.vs.Mock.A549.2$Count,decreasing = T)][1:10]
goUP.SARS.vs.Mock.A549.2

## ID
## GO:0010498 GO:0010498
## GO:0022604 GO:0022604
## GO:0043087 GO:0043087
## GO:0043161 GO:0043161
## GO:0006605 GO:0006605
## GO:0016311 GO:0016311
## GO:0010256 GO:0010256
## GO:0009896 GO:0009896
## GO:0007409 GO:0007409
## GO:0043254 GO:0043254

## Description
## GO:0010498 proteasomal protein catabolic process
## GO:0022604 regulation of cell morphogenesis
## GO:0043087 regulation of GTPase activity
## GO:0043161 proteasome-mediated ubiquitin-dependent protein catabolic process
## GO:0006605 protein targeting
## GO:0016311 dephosphorylation
## GO:0010256 endomembrane system organization
## GO:0009896 positive regulation of catabolic process
## GO:0007409 axonogenesis
## GO:0043254 regulation of protein-containing complex assembly

## GeneRatio BgRatio pvalue p.adjust qvalue
## GO:0010498 166/3905 483/18866 1.124177e-12 1.252629e-09 1.071975e-09
## GO:0022604 151/3905 499/18866 2.108685e-07 3.351755e-05 2.868367e-05
## GO:0043087 148/3905 481/18866 9.033769e-08 1.728514e-05 1.479229e-05
## GO:0043161 146/3905 424/18866 2.225914e-11 1.179363e-08 1.009276e-08
## GO:0006605 146/3905 444/18866 8.999884e-10 3.365957e-07 2.880520e-07
## GO:0016311 145/3905 492/18866 1.930696e-06 1.859904e-04 1.591670e-04
## GO:0010256 144/3905 468/18866 1.344599e-07 2.310530e-05 1.977307e-05
## GO:0009896 143/3905 454/18866 3.031510e-08 6.646324e-06 5.687794e-06
## GO:0007409 143/3905 482/18866 1.514790e-06 1.660523e-04 1.421043e-04
## GO:0043254 137/3905 449/18866 4.569201e-07 6.315431e-05 5.404623e-05

## GeneRatio BgRatio pvalue p.adjust qvalue
## GO:0010498 57761/1649/64750/8473/5366/4734/7328/23392/7324/91147/4189/9306/55757/4287/54431/4780/4779
## GO:0022604
## GO:0043087
## GO:0043161
## GO:0006605
## GO:0016311
## GO:0010256
## GO:0009896
## GO:0007409

```

```

## GO:0043254
##          Count
## GO:0010498    166
## GO:0022604    151
## GO:0043087    148
## GO:0043161    146
## GO:0006605    146
## GO:0016311    145
## GO:0010256    144
## GO:0009896    143
## GO:0007409    143
## GO:0043254    137

```

GO enrichment for SARS-CoV-2_vs_Mock in A549.ACE2

```

updiff <- subset(R.SARS.vs.Mock.A549ACE2.2, logFC > 0 & adj.P.Val < 0.05)
upgene <- rownames(updiff)
gene_name=as.vector(upgene)
geneID <- mget(gene_name, org.Hs.egSYMBOL2EG, ifnotfound=NA)%>%as.character()
data=cbind(Gene=upgene, entrezID=geneID)%>%as.data.frame()
go.SARS.vs.Mock.A549.ACE2.2 <- enrichGO(gene=data$entrezID, OrgDb = org.Hs.eg.db, ont='BP', pvalueCutoff
goUP.SARS.vs.Mock.A549.ACE2.2 <- go.SARS.vs.Mock.A549.ACE2.2[order(go.SARS.vs.Mock.A549.ACE2.2$Count, de
goUP.SARS.vs.Mock.A549.ACE2.2

```

	ID	Description				
##	## GO:0016569	GO:0016569 covalent chromatin modification				
##	## GO:0016570	GO:0016570 histone modification				
##	## GO:0008380	GO:0008380 RNA splicing				
##	## GO:0018205	GO:0018205 peptidyl-lysine modification				
##	## GO:0000375	GO:0000375 RNA splicing, via transesterification reactions				
##	## GO:1903706	GO:1903706 regulation of hemopoiesis				
##	## GO:0000377	GO:0000377 RNA splicing, via transesterification reactions with bulged adenosine as nucleophile				
##	## GO:0000398	GO:0000398 mRNA splicing, via spliceosome				
##	## GO:0009896	GO:0009896 positive regulation of catabolic process				
##	## GO:0051090	GO:0051090 regulation of DNA-binding transcription factor activity				
##	GeneRatio	BgRatio	pvalue	p.adjust	qvalue	
##	## GO:0016569	219/4002	481/18866	1.300515e-33	8.249169e-30	6.625784e-30
##	## GO:0016570	213/4002	468/18866	1.102249e-32	3.495784e-29	2.807835e-29
##	## GO:0008380	212/4002	487/18866	3.149427e-29	6.658939e-26	5.348501e-26
##	## GO:0018205	172/4002	405/18866	1.728405e-22	2.740818e-19	2.201442e-19
##	## GO:0000375	165/4002	393/18866	5.273639e-21	6.690138e-18	5.373560e-18
##	## GO:1903706	165/4002	498/18866	2.691076e-10	2.404154e-08	1.931031e-08
##	## GO:0000377	163/4002	390/18866	1.586567e-20	1.437656e-17	1.154735e-17

```

## GO:0000398 163/4002 390/18866 1.586567e-20 1.437656e-17 1.154735e-17
## GO:0009896 160/4002 454/18866 2.284334e-12 3.813035e-10 3.062653e-10
## GO:0051090 154/4002 455/18866 1.887512e-10 1.735143e-08 1.393678e-08
##
## GO:0016569 23135/5187/200424/3976/80314/51742/200186/201163/6018/55904/80853/10765/57708/9958/51773/
## GO:0016570 23135/5187/200424/3976/80314/51742/200186/201163/6018/
## GO:0008380 51585/5411/1195/9589/84081/91603/9584/8106/79753/10658/6651,
## GO:0018205
## GO:0000375
## GO:1903706
## GO:0000377
## GO:0000398
## GO:0009896
## GO:0051090
##          Count
## GO:0016569 219
## GO:0016570 213
## GO:0008380 212
## GO:0018205 172
## GO:0000375 165
## GO:1903706 165
## GO:0000377 163
## GO:0000398 163
## GO:0009896 160
## GO:0051090 154

```

GO enrichment for SARS-CoV-2_vs_Mock in Calu-3

```

updiff <- subset(R.SARS.vs.Mock.Calu3, logFC > 0 & adj.P.Val < 0.05)
upgene <- rownames(updiff)
gene_name=as.vector(upgene)
geneID <- mget(gene_name, org.Hs.egSYMBOL2EG, ifnotfound=NA)%>%as.character()
data=cbind(Gene=upgene,entrezID= geneID)%>%as.data.frame()
go.SARS.vs.Mock.Calu3 <- enrichGO(gene=data$entrezID, OrgDb = org.Hs.eg.db, ont='BP',pvalueCutoff = 0.05)
goUP.SARS.vs.Mock.Calu3 <- go.SARS.vs.Mock.Calu3[order(go.SARS.vs.Mock.Calu3$Count,decreasing = T)][1:10]
goUP.SARS.vs.Mock.Calu3

##           ID                               Description
## GO:1903706 GO:1903706 regulation of hemopoiesis
## GO:0051090 GO:0051090 regulation of DNA-binding transcription factor activity
## GO:0001819 GO:0001819 positive regulation of cytokine production
## GO:0009896 GO:0009896 positive regulation of catabolic process
## GO:0016569 GO:0016569 covalent chromatin modification
## GO:0009615 GO:0009615 response to virus
## GO:0022407 GO:0022407 regulation of cell-cell adhesion
## GO:0016570 GO:0016570 histone modification
## GO:0045785 GO:0045785 positive regulation of cell adhesion
## GO:0043087 GO:0043087 regulation of GTPase activity
##           GeneRatio   BgRatio      pvalue     p.adjust    qvalue
## GO:1903706 150/3258 498/18866 6.410525e-13 1.743105e-10 1.320245e-10
## GO:0051090 148/3258 455/18866 8.477041e-16 8.835903e-13 6.692401e-13
## GO:0001819 141/3258 447/18866 6.394527e-14 2.666091e-11 2.019324e-11
## GO:0009896 141/3258 454/18866 2.452540e-13 7.669092e-11 5.808647e-11
## GO:0016569 139/3258 481/18866 1.166066e-10 1.519287e-08 1.150723e-08

```

```

## GO:0009615 136/3258 349/18866 2.575425e-22 8.053354e-19 6.099691e-19
## GO:0022407 135/3258 439/18866 1.779536e-12 4.280468e-10 3.242070e-10
## GO:0016570 135/3258 468/18866 2.467367e-10 2.911493e-08 2.205194e-08
## GO:0045785 134/3258 428/18866 5.014625e-13 1.425845e-10 1.079949e-10
## GO:0043087 133/3258 481/18866 6.450182e-09 5.171723e-07 3.917115e-07
##
## GO:1903706 3552/4792/3553/3659/3624/56892/7538/22806/7071/3665/7057/1437/6772/3965/345
## GO:0051090 4792/3383/3569/7128/3553/10346/6376/23586/6672/3656/9314/3965/7185/84166/6737/85363/3725/
## GO:0001819 91543/3552/64135/3569/3553,
## GO:0009896 3569/7128/3553/25976/10346/7538/56829/151636/154/87178/10133/6737/85363/4734/6662/153
## GO:0016569 3553/151636/3976/7422/5187/23135/9734/80853/122953/80314/74
## GO:0009615 3433/4939/3434/3437/91543
## GO:0022407
## GO:0016570 3553/151636/3976/7422/5187/23135/9734
## GO:0045785
## GO:0043087 3383/6364/6352/6376/25780/6347/3725/4739
##
## Count
## GO:1903706 150
## GO:0051090 148
## GO:0001819 141
## GO:0009896 141
## GO:0016569 139
## GO:0009615 136
## GO:0022407 135
## GO:0016570 135
## GO:0045785 134
## GO:0043087 133

```

GO enrichment for IAV_vs_Mock in NHBE

```

updiff <- subset(R.IAV.vs.Mock.nhbe, logFC > 0 & adj.P.Val < 0.05)
upgene <- rownames(updiff)
gene_name=as.vector(upgene)
geneID <- mget(gene_name, org.Hs.egSYMBOL2EG, ifnotfound=NA)%>%as.character()
data=cbind(Gene=upgene,entrezID=GeneID)%>%as.data.frame()
go.IAV.vs.Mock.nhbe<- enrichGO(gene=data$entrezID, OrgDb = org.Hs.eg.db, ont='BP', pvalueCutoff = 0.05)%
goUP.IAV.vs.Mock.nhbe <- go.IAV.vs.Mock.nhbe[order(go.IAV.vs.Mock.nhbe$Count,decreasing = T)[1:10],]
goUP.IAV.vs.Mock.nhbe

```

	ID	Description	GeneRatio	
## GO:0009615	GO:0009615	response to virus	79/1560	
## GO:0043087	GO:0043087	regulation of GTPase activity	77/1560	
## GO:0009896	GO:0009896	positive regulation of catabolic process	76/1560	
## GO:0010256	GO:0010256	endomembrane system organization	74/1560	
## GO:1901653	GO:1901653	cellular response to peptide	73/1560	
## GO:0043434	GO:0043434	response to peptide hormone	73/1560	
## GO:0010498	GO:0010498	proteasomal protein catabolic process	73/1560	
## GO:0016569	GO:0016569	covalent chromatin modification	72/1560	
## GO:0042326	GO:0042326	negative regulation of phosphorylation	72/1560	
## GO:0050769	GO:0050769	positive regulation of neurogenesis	72/1560	
##	BgRatio	pvalue	p.adjust	qvalue
## GO:0009615	349/18866	7.906076e-17	2.300668e-13	1.736008e-13
## GO:0043087	481/18866	1.320797e-08	2.454787e-06	1.852301e-06
## GO:0009896	454/18866	2.217125e-09	5.610291e-07	4.233340e-07

```

## GO:0010256 468/18866 4.274958e-08 6.005468e-06 4.531527e-06
## GO:1901653 398/18866 6.914994e-11 4.024527e-08 3.036774e-08
## GO:0043434 447/18866 1.349711e-08 2.454787e-06 1.852301e-06
## GO:0010498 483/18866 3.275439e-07 2.845232e-05 2.146917e-05
## GO:0016569 481/18866 5.720924e-07 4.689546e-05 3.538576e-05
## GO:0042326 484/18866 7.258249e-07 5.558290e-05 4.194102e-05
## GO:0050769 485/18866 7.851877e-07 5.641719e-05 4.257054e-05
##
## GO:0009615 7128/8780/4940/10379/1191/103/5611/151636/330/85363/1021/9188/6352/4599/91543/5610/54765/
## GO:0043087      3688/25782/57186/3383/6352/3655/5048/23365/10253/3843/1121/6102/10565/85440/55785/430
## GO:0009896 9709/7128/8754/3320/140775/253943/1191/9475/25782/151636/85363/54467/6737/6093/6653/92140,
## GO:0010256          196527/9451/23271/1191/10564/25782/488/5660/23592/5048/23122/26092/3998
## GO:1901653          64397/113/11031/9475/5576/7071/6774/3383/5048
## GO:0043434          64397/113/11031/9475/5576/6774/3383/5048
## GO:0010498          9709/6400/7184/1649/7706/9572/3309/1191/54467/23291/7328/11160/54431/1499
## GO:0016569          55196/9329/151636/9320/5927/25836/221656/8202/1499/5187/23429/221656
## GO:0042326          7128/140775/103/5611/5576/387/6774/135228/5048
## GO:0050769          23064/3309/9043/3572/387/1435/3655/5048
##
##           Count
## GO:0009615    79
## GO:0043087    77
## GO:0009896    76
## GO:0010256    74
## GO:1901653    73
## GO:0043434    73
## GO:0010498    73
## GO:0016569    72
## GO:0042326    72
## GO:0050769    72

intf.IAV <- go.IAV.vs.Mock.nhbe %>%
  select(ID,Description,GeneRatio,BgRatio,pvalue,p.adjust,qvalue,geneID,Count) %>%
  filter(str_detect>Description,"type I interferon"))
intf.IAV

##           ID
## GO:0034340 GO:0034340
## GO:0060337 GO:0060337
## GO:0071357 GO:0071357
## GO:0032606 GO:0032606
## GO:0032479 GO:0032479
## GO:0032481 GO:0032481
## GO:0060338 GO:0060338
## GO:0060339 GO:0060339
## GO:0032480 GO:0032480
##
##           Description
## GO:0034340      response to type I interferon
## GO:0060337      type I interferon signaling pathway
## GO:0071357      cellular response to type I interferon
## GO:0032606      type I interferon production
## GO:0032479      regulation of type I interferon production
## GO:0032481      positive regulation of type I interferon production
## GO:0060338      regulation of type I interferon-mediated signaling pathway
## GO:0060339      negative regulation of type I interferon-mediated signaling pathway
## GO:0032480      negative regulation of type I interferon production

```

```

##          GeneRatio   BgRatio      pvalue      p.adjust      qvalue
## GO:0034340    31/1560 99/18866 3.685154e-11 3.574600e-08 2.697274e-08
## GO:0060337    30/1560 95/18866 6.001447e-11 3.880936e-08 2.928426e-08
## GO:0071357    30/1560 95/18866 6.001447e-11 3.880936e-08 2.928426e-08
## GO:0032606    33/1560 127/18866 2.010541e-09 5.318794e-07 4.013386e-07
## GO:0032479    31/1560 125/18866 2.034989e-08 3.200983e-06 2.415355e-06
## GO:0032481    19/1560 74/18866 6.272518e-06 2.786722e-04 2.102768e-04
## GO:0060338    10/1560 35/18866 3.896841e-04 5.752767e-03 4.340847e-03
## GO:0060339      6/1560 15/18866 8.238388e-04 9.968279e-03 7.521732e-03
## GO:0032480    11/1560 47/18866 1.282405e-03 1.354955e-02 1.022404e-02
##
## GO:0034340           4940/253943/10379/103/4599/91543/6672/3106/5781/3434/3665/3437/6772/3433/8
## GO:0060337           4940/253943/10379/103/4599/91543/6672/3106/5781/3434/3665/3437/6772/7
## GO:0071357           4940/253943/10379/103/4599/91543/6672/3106/5781/3434/3665/3437/6772/6
## GO:0032606 7128/8780/7706/56919/6737/5781/51191/1499/83737/1540/11128/3665/6772/23586/1654/9636/1705
## GO:0032479           7128/8780/7706/56919/6737/5781/51191/1499/83737/1540/11128/3665/6772/23586/1654
## GO:0032481
## GO:0060338
## GO:0060339
## GO:0032480
##          Count
## GO:0034340    31
## GO:0060337    30
## GO:0071357    30
## GO:0032606    33
## GO:0032479    31
## GO:0032481    19
## GO:0060338    10
## GO:0060339      6
## GO:0032480    11

chemok.IAV <- go.IAV.vs.Mock.nhbe %>%
  select(ID,Description,GeneRatio,BgRatio,pvalue,p.adjust,qvalue,geneID,Count) %>%
  filter(str_detect>Description,"chemokine"))
chemok.IAV

## [1] ID          Description GeneRatio   BgRatio      pvalue      p.adjust
## [7] qvalue      geneID      Count
## <0 rows> (or 0-length row.names)

cytokine.IAV <- go.IAV.vs.Mock.nhbe %>%
  select(ID,Description,GeneRatio,BgRatio,pvalue,p.adjust,qvalue,geneID,Count) %>%
  filter(str_detect>Description,"cytokine"))
cytokine.IAV

##
##          ID
## GO:0060759 GO:0060759
## GO:0001959 GO:0001959
## GO:0001819 GO:0001819
## GO:0060760 GO:0060760
## GO:0001961 GO:0001961
## GO:0061640 GO:0061640
## GO:0000910 GO:0000910
## GO:0000281 GO:0000281
##                                     Description GeneRatio
## GO:0060759 regulation of response to cytokine stimulus 45/1560

```

```

## GO:0001959 regulation of cytokine-mediated signaling pathway 42/1560
## GO:0001819 positive regulation of cytokine production 65/1560
## GO:0060760 positive regulation of response to cytokine stimulus 16/1560
## GO:0001961 positive regulation of cytokine-mediated signaling pathway 14/1560
## GO:0061640 cytoskeleton-dependent cytokinesis 20/1560
## GO:0000910 cytokinesis 29/1560
## GO:0000281 mitotic cytokinesis 14/1560
##          BgRatio      pvalue     p.adjust      qvalue
## GO:0060759 196/18866 2.198370e-10 9.841935e-08 7.426397e-08
## GO:0001959 183/18866 8.825524e-10 3.021444e-07 2.279881e-07
## GO:0001819 447/18866 5.399565e-06 2.474446e-04 1.867135e-04
## GO:0060760 57/18866 9.789495e-06 4.040770e-04 3.049031e-04
## GO:0001961 50/18866 3.600292e-05 1.068496e-03 8.062518e-04
## GO:0061640 100/18866 1.721352e-04 3.296391e-03 2.487347e-03
## GO:0000910 172/18866 1.758657e-04 3.355863e-03 2.532223e-03
## GO:0000281 72/18866 2.061362e-03 1.922617e-02 1.450743e-02
##
## GO:0060759
## GO:0001959
## GO:0001819 1649/8780/9451/7057/1191/10564/9475/56919/330/3572/6774/9188/5295/91543/29126/6653/5781/5
## GO:0060760
## GO:0001961
## GO:0061640
## GO:0000910
## GO:0000281
##          Count
## GO:0060759    45
## GO:0001959    42
## GO:0001819    65
## GO:0060760    16
## GO:0001961    14
## GO:0061640    20
## GO:0000910    29
## GO:0000281    14

inflammatory.IAV <- go.IAV.vs.Mock.nhbe %>%
  select(ID,Description,GeneRatio,BgRatio,pvalue,p.adjust,qvalue,geneID,Count) %>%
  filter(str_detect>Description,"inflammatory"))
inflammatory.IAV

##          ID                  Description GeneRatio BgRatio
## GO:0006925 GO:0006925 inflammatory cell apoptotic process 8/1560 22/18866
##          pvalue     p.adjust      qvalue
## GO:0006925 0.0002390144 0.004105546 0.003097909
##          geneID Count
## GO:0006925 6352/23657/3665/5291/3569/10269/6950/23411     8

IAV.dif <- rbind(intf.IAV,chemok.IAV,cytokine.IAV,inflammatory.IAV)
IAV.dif <- subset(IAV.dif, p.adjust<0.001 )
IAV.dif

##          ID                  Description
## GO:0034340 GO:0034340 response to type I interferon
## GO:0060337 GO:0060337 type I interferon signaling pathway
## GO:0071357 GO:0071357 cellular response to type I interferon
## GO:0032606 GO:0032606 type I interferon production

```

```

## GO:0032479 GO:0032479      regulation of type I interferon production
## GO:0032481 GO:0032481  positive regulation of type I interferon production
## GO:0060759 GO:0060759      regulation of response to cytokine stimulus
## GO:0001959 GO:0001959  regulation of cytokine-mediated signaling pathway
## GO:0001819 GO:0001819      positive regulation of cytokine production
## GO:0060760 GO:0060760 positive regulation of response to cytokine stimulus
##           GeneRatio   BgRatio      pvalue     p.adjust    qvalue
## GO:0034340  31/1560  99/18866 3.685154e-11 3.574600e-08 2.697274e-08
## GO:0060337  30/1560  95/18866 6.001447e-11 3.880936e-08 2.928426e-08
## GO:0071357  30/1560  95/18866 6.001447e-11 3.880936e-08 2.928426e-08
## GO:0032606  33/1560  127/18866 2.010541e-09 5.318794e-07 4.013386e-07
## GO:0032479  31/1560  125/18866 2.034989e-08 3.200983e-06 2.415355e-06
## GO:0032481  19/1560  74/18866 6.272518e-06 2.786722e-04 2.102768e-04
## GO:0060759  45/1560  196/18866 2.198370e-10 9.841935e-08 7.426397e-08
## GO:0001959  42/1560  183/18866 8.825524e-10 3.021444e-07 2.279881e-07
## GO:0001819  65/1560  447/18866 5.399565e-06 2.474446e-04 1.867135e-04
## GO:0060760  16/1560  57/18866 9.789495e-06 4.040770e-04 3.049031e-04
##
## GO:0034340
## GO:0060337
## GO:0071357
## GO:0032606
## GO:0032479
## GO:0032481
## GO:0060759
## GO:0001959
## GO:0001819 1649/8780/9451/7057/1191/10564/9475/56919/330/3572/6774/9188/5295/91543/29126/6653/5781/50
## GO:0060760
##           Count
## GO:0034340  31
## GO:0060337  30
## GO:0071357  30
## GO:0032606  33
## GO:0032479  31
## GO:0032481  19
## GO:0060759  45
## GO:0001959  42
## GO:0001819  65
## GO:0060760  16

```

Visualization for GO enrichment result:

```

goUP.IAV.vs.Mock.nhbe$p.adjust <- -log10(goUP.IAV.vs.Mock.nhbe$p.adjust)
goUP.IAV.vs.Mock.nhbe$GeneRatio <- goUP.IAV.vs.Mock.nhbe$Count/1560*100

# goUP.IAV.vs.Mock.A549$p.adjust <- -log10(goUP.IAV.vs.Mock.A549$p.adjust)
# goUP.IAV.vs.Mock.A549$GeneRatio <- goUP.IAV.vs.Mock.A549$Count/1999*100

goUP.IFNB12h.vs.Mock.nhbe$p.adjust <- -log10(goUP.IFNB12h.vs.Mock.nhbe$p.adjust)
goUP.IFNB12h.vs.Mock.nhbe$GeneRatio <- goUP.IFNB12h.vs.Mock.nhbe$Count/1283*100

```

```

goUP.IFN6h.vs.Mock.nhbe$p.adjust <- -log10(goUP.IFN6h.vs.Mock.nhbe$p.adjust)
goUP.IFN6h.vs.Mock.nhbe$GeneRatio <- goUP.IFN6h.vs.Mock.nhbe$Count/2021*100
goUP.IFN4h.vs.Mock.nhbe$p.adjust <- -log10(goUP.IFN4h.vs.Mock.nhbe$p.adjust)
goUP.IFN4h.vs.Mock.nhbe$GeneRatio <- goUP.IFN4h.vs.Mock.nhbe$Count/1188*100

goUP.SARS.vs.Mock.nhbe$p.adjust <- -log10(goUP.SARS.vs.Mock.nhbe$p.adjust)
goUP.SARS.vs.Mock.nhbe$GeneRatio <- goUP.SARS.vs.Mock.nhbe$Count/241*100

intf.IAV <- subset(intf.IAV, p.adjust<0.001 )
intf.SARS <- subset(intf.SARS, p.adjust<0.001 )
intf.IAV$p.adjust <- -log10(intf.IAV$p.adjust)
intf.IAV$GeneRatio <- intf.IAV$Count/1560*100
intf.SARS$p.adjust <- -log10(intf.SARS$p.adjust)
intf.SARS$GeneRatio <- intf.SARS$Count/241*100

```

Dotplot for different treatment in NHBE

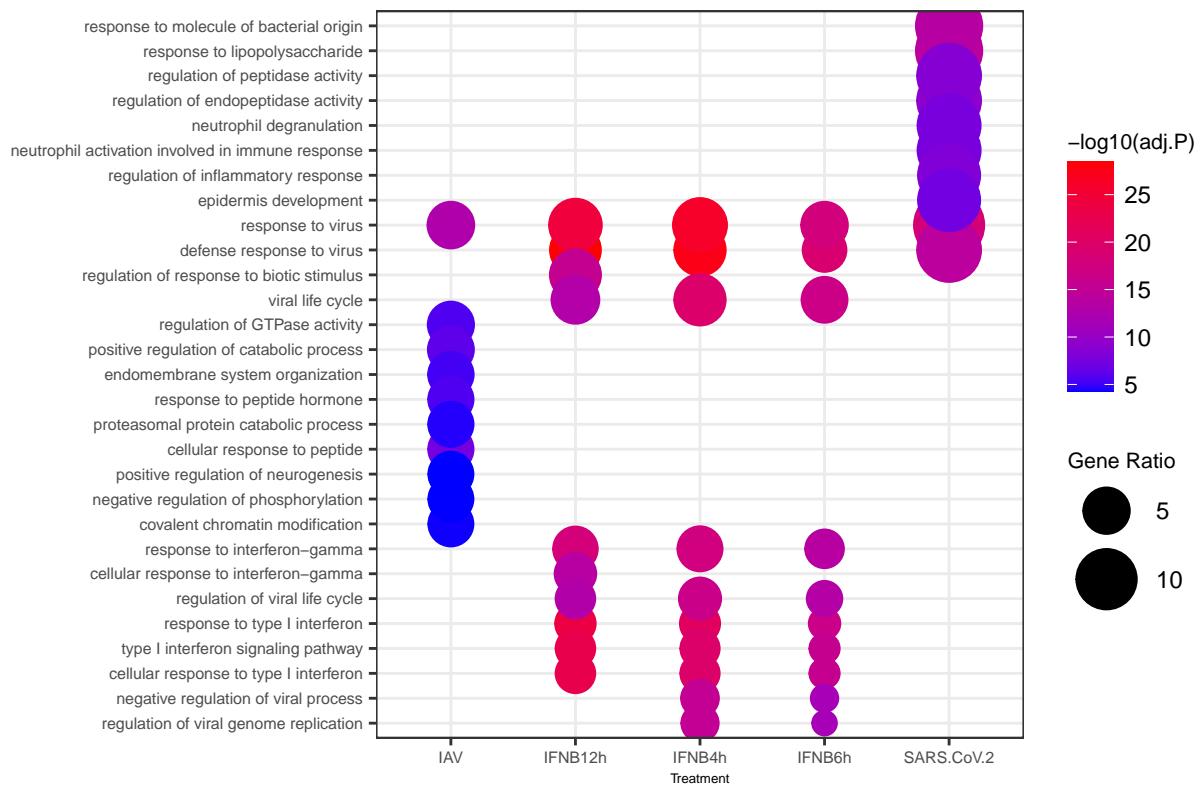
```

go1<- rbind(goUP.IFN12h.vs.Mock.nhbe,goUP.IFN6h.vs.Mock.nhbe,
             goUP.IFN4h.vs.Mock.nhbe,goUP.IAV.vs.Mock.nhbe,
             goUP.SARS.vs.Mock.nhbe)
go1$combination <- c(rep("IFN12h",10),rep("IFN6h",10),rep("IFN4h",10),
                      rep("IAV",10),
                      rep("SARS.Cov.2",10))

ggplot(go1,aes(combination,reorder>Description,GeneRatio),color=p.adjust,size=GeneRatio))+ 
  geom_point(shape=19)+ 
  scale_color_gradient(low = "blue",high = "red")+
  scale_size_continuous(range = c(4,12))+ 
  labs(x="Treatment",y="",color="-log10(adj.P)",size="Gene Ratio")+
  ggtitle("Top 10 GO enrichment of different treatment in NHBE")+
  theme_bw()+
  theme(axis.title = element_text(size = 5),
        axis.text = element_text(size = 6),
        legend.title = element_text(size = 8),
        plot.title = element_text(size = 18,face = "bold",hjust = 0.5))

```

Top 10 GO enrichment of different treatment in NF



```
goUP.SARS.vs.Mock.A549.2$p.adjust <- -log10(goUP.SARS.vs.Mock.A549.2$p.adjust)
goUP.SARS.vs.Mock.A549.2$GeneRatio <- goUP.SARS.vs.Mock.A549.2$Count/3905*100
```

```
goUP.SARS.vs.Mock.A549.ACE2.2$p.adjust <- -log10(goUP.SARS.vs.Mock.A549.ACE2.2$p.adjust)
goUP.SARS.vs.Mock.A549.ACE2.2$GeneRatio <- goUP.SARS.vs.Mock.A549.ACE2.2$Count/4002*100
```

```
goUP.SARS.vs.Mock.Calu3$p.adjust <- -log10(goUP.SARS.vs.Mock.Calu3$p.adjust)
goUP.SARS.vs.Mock.Calu3$GeneRatio <- goUP.SARS.vs.Mock.Calu3$Count/3258*100
```

Dotplot for 4 SARS.Cov.2 infected cells

```
go1<- rbind(goUP.SARS.vs.Mock.A549.2,goUP.SARS.vs.Mock.A549.ACE2.2,
              goUP.SARS.vs.Mock.Calu3,goUP.SARS.vs.Mock.nhbe)
go1$Cell.line <- c(rep("A549",10),
                    rep("A549.ACE2",10),
                    rep("Calu-3",10),
                    rep("NHBE",10))
```

```
ggplot(go1,aes(Cell.line,reorder(Description,GeneRatio),color=p.adjust,size=GeneRatio))+  
  geom_point(shape=19)+
```

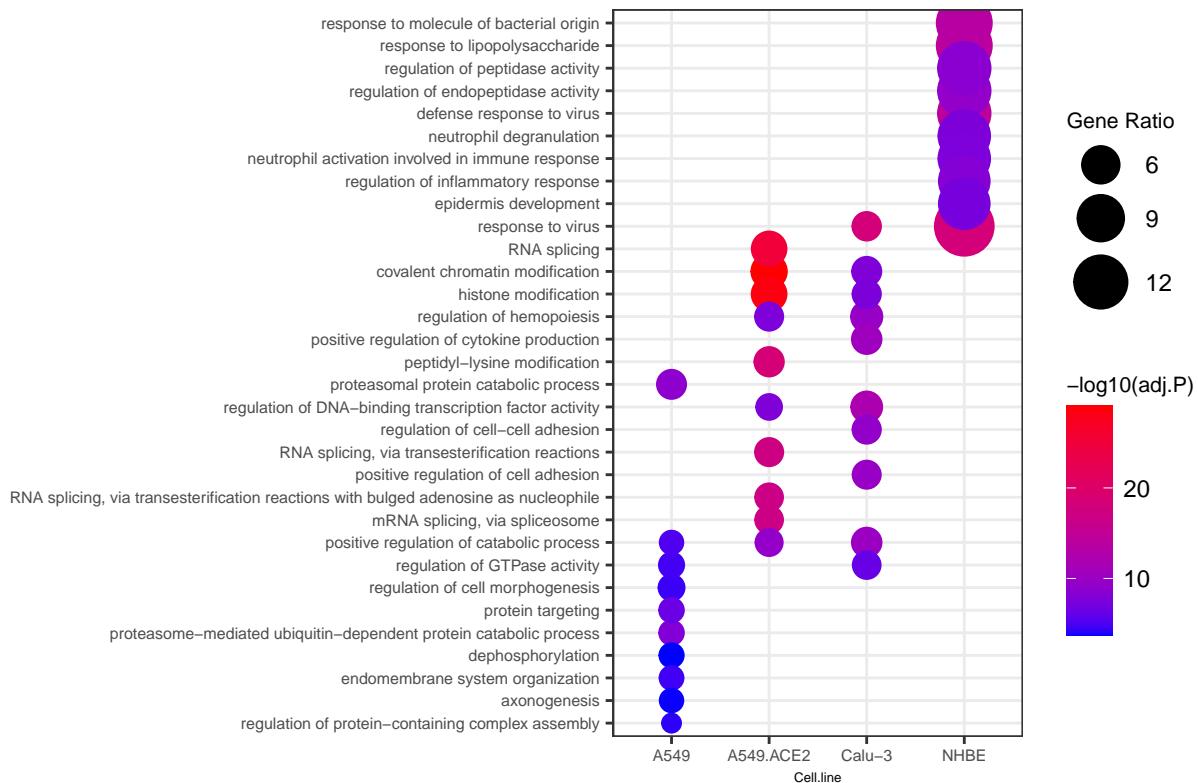
```

scale_color_gradient(low = "blue",high = "red")+
scale_size_continuous(range = c(3,10))+  

labs(x="Cell.line",y="",color="-log10(adj.P)",size="Gene Ratio")+
ggtitle("Top10 GO enrichment of 4 SARS.Cov.2 infected cells")+
theme_bw()+
theme(axis.title = element_text(size = 5),
axis.text = element_text(size = 6),
legend.title = element_text(size = 8),
plot.title = element_text(size = 18,face = "bold",hjust = 0.5))

```

Top10 GO enrichment of 4 SARS.Cov.2 infec



Dotplot for SARS.Cov.2/IAV in NHBE

```

IAV.dif$p.adjust <- -log10(IAV.dif$p.adjust)
IAV.dif$GeneRatio <- IAV.dif$Count/1560*100

SARS.dif$p.adjust <- -log10(SARS.dif$p.adjust)
SARS.dif$GeneRatio <- SARS.dif$Count/241*100

```

```

go1<- rbind(goUP.IAV.vs.Mock.nhbe,
              goUP.SARS.vs.Mock.nhbe)
go1$Cell.line <- c(rep("NHBE_IAV",10),
                   rep("NHBE_SARS.CoV.2",10))

```

```

ggplot(go1,aes(Cell.line,reorder(Description,GeneRatio),color=p.adjust,size=GeneRatio))+  

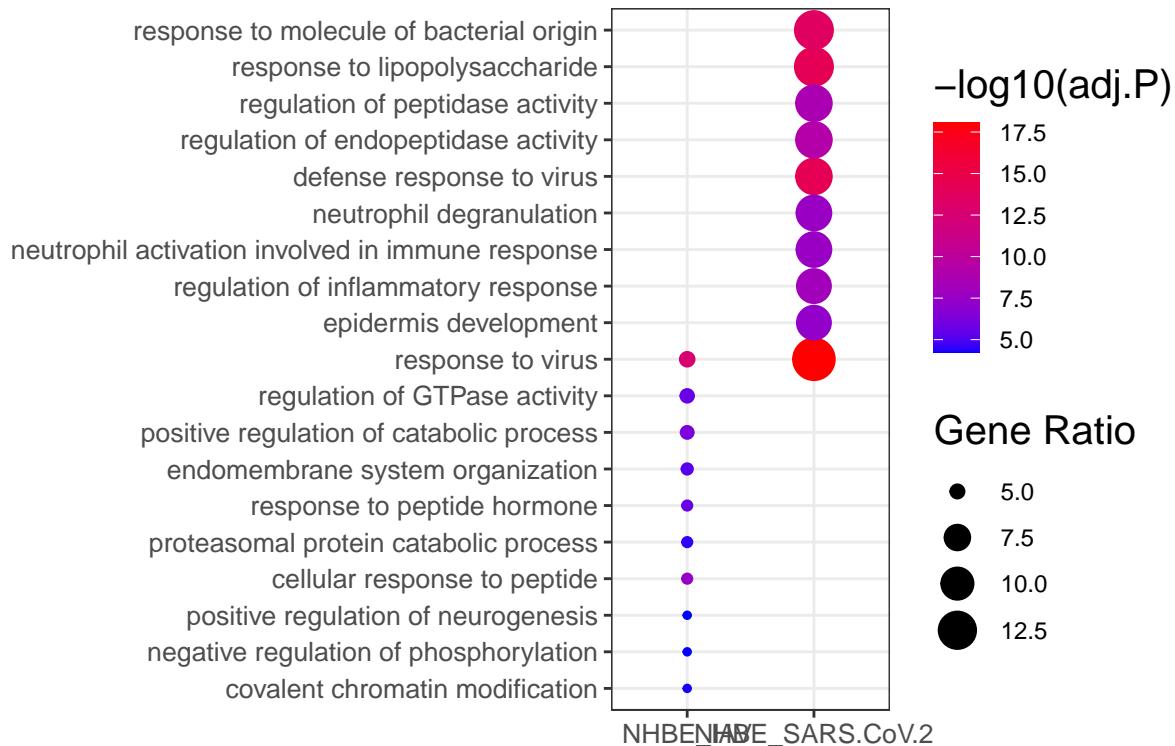
  geom_point(shape=19)+  

  scale_color_gradient(low = "blue",high = "red")+
  scale_size_continuous(range = c(1,7))+  

  labs(x="",y="",color="-log10(adj.P)",size="Gene Ratio")+
  ggtitle("Top 10 GO enrichment of SARS.Cov.2/IAV in NHBE")+
  theme_bw()+
  theme(axis.title = element_text(size = 14),
        axis.text = element_text(size = 10),
        legend.title = element_text(size = 15),
        plot.title = element_text(size = 15,face = "bold",hjust = 0.5))

```

Top 10 GO enrichment of SARS.Cov.2/IAV in NHB



```

go1<- rbind(IAV.tif,SARS.tif)
go1$Cell.line <- c(rep("NHBE_IAV",nrow(IAV.tif)),
                    rep("NHBE_SARS_CoV.2",nrow(SARS.tif)))

```



```

ggplot(go1,aes(Cell.line,reorder(Description,GeneRatio),color=p.adjust,size=GeneRatio))+  

  geom_point(shape=19)+  

  scale_color_gradient(low = "blue",high = "red")+
  scale_size_continuous(range = c(3,10))+  

  labs(x="",y="",color="-log10(adj.P)",size="Gene Ratio")+
  ggtitle("GO enrichment of SARS.Cov.2/IAV in NHBE")+
  theme_bw()+
  theme(axis.title = element_text(size = 5),
        axis.text = element_text(size = 6),
        legend.title = element_text(size = 8),

```

```
plot.title = element_text(size = 18, face = "bold", hjust = 0.5))
```

GO enrichment of SARS.Cov.2/IAV in NHBE

