Case-Study-for-ADO-PCE-1: Global Gene Expression of 2B4 Cells in Response to SARS-CoV Infection

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Contents

The last versión of the current document, with all the relevant files and code, and all data associated with the report can be found in:

https://github.com/piosierra/ADO-PEC1

The original study can be found in:

https://www.ncbi.nlm.nih.gov/pubmed/20090954

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1 Abstract

We reproduce the microarray analysis of the study *Dynamic Innate Immune Responses of Human Bronchial Epithelial Cells to Severe Acute Respiratory Syndrome-Associated Coronavirus Infection* **(Yoshikawa et al. 2010)**. They employed a cDNA microarray to analyze the patterns of the global gene expression of 2B4 cells in response to SARS-CoV, as the first step to explore the likely antiviral signaling pathway/s.

2 Objectives

To perform a differential gene expression (DGE) analysis on SARS-CoV infected 2B4 cells at 12-, 24-, and 48-hrs post infection. The study aims to identify the complex epithelial signaling to SARS-CoV that is crucial for paving the way to better understand SARS pathogenesis.

As mentioned on the study, even if the analysis was performed simultaneosly for the Sars-CoV and DOHV infected cells, for the study they did only use the files belonging to the control and Sars-Cov infected cells. The analysis with the DOHV data belongs to a different study and it is not included on that one.

3 Materials and Methods

3.1 Materials

We use the published data for the experiment that can be found on GEO GSE17400:
https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE17400

We will reproduce the whole experiment, again, even if the DOHV data was not used in the mentioned study. To characterize the dynamic, spatial, and temporal changes of the gene expression induced by SARS-CoV, confluent 2B4 cells grown in T-75 flasks were infected with SARS-CoV (MOI=0.1) or remained uninfected (as control) for 12, 24, and 48hrs. Because 2B4 cells were also permissive to the productive infection of Dhori virus (DHOV), a member of the Orthomyxoviridae family within the Thogotovirus genus, resulting in robust responses of IFNs and other pro-inflammatory mediators, they also established parallel cultures of DHOV-infected 2B4 cells (MOI=0.1) for the comparative analysis of global gene expression elicited by SARS-CoV- versus DHOV-infected 2B4 cells. To meet the minimal number required for application of statistical algorithms, we performed the study in triplicate at each time point for mock-, SARS-CoV-, and DHOV-infected cultures, yielding a total of 27 arrays. Mock-infected cells were compared to cells infected with SARS-CoV or DHOV at each time point.

The microarrays used were Affymetrix Human Genome U133 Plus 2.0 Array annotation data (chip hgu133plus2)
http://bioconductor.org/packages/release/data/annotation/html/hgu133plus2.db.html
https://www.affymetrix.com/support/technical/datasheets/human_datasheet.pdf

We used R and Bioconductor to perform the analysis. The full list of packages employed can be found on the Apnedix.

3.2 Methods

On the working directory it is asumed we have the following folders to work with: - data: where the CEL files are downloaded
- results: where the results data files will be stored
- figures: where the figures produced are stored

We describe now the workflow followed:

a) Identify the groups and match groups to files.

There are 27 arrays were analyzed as 9 separate groups (mock-, SARS-CoV-infected, and DHOV-infected cells at 12, 24, and 48 hrs).

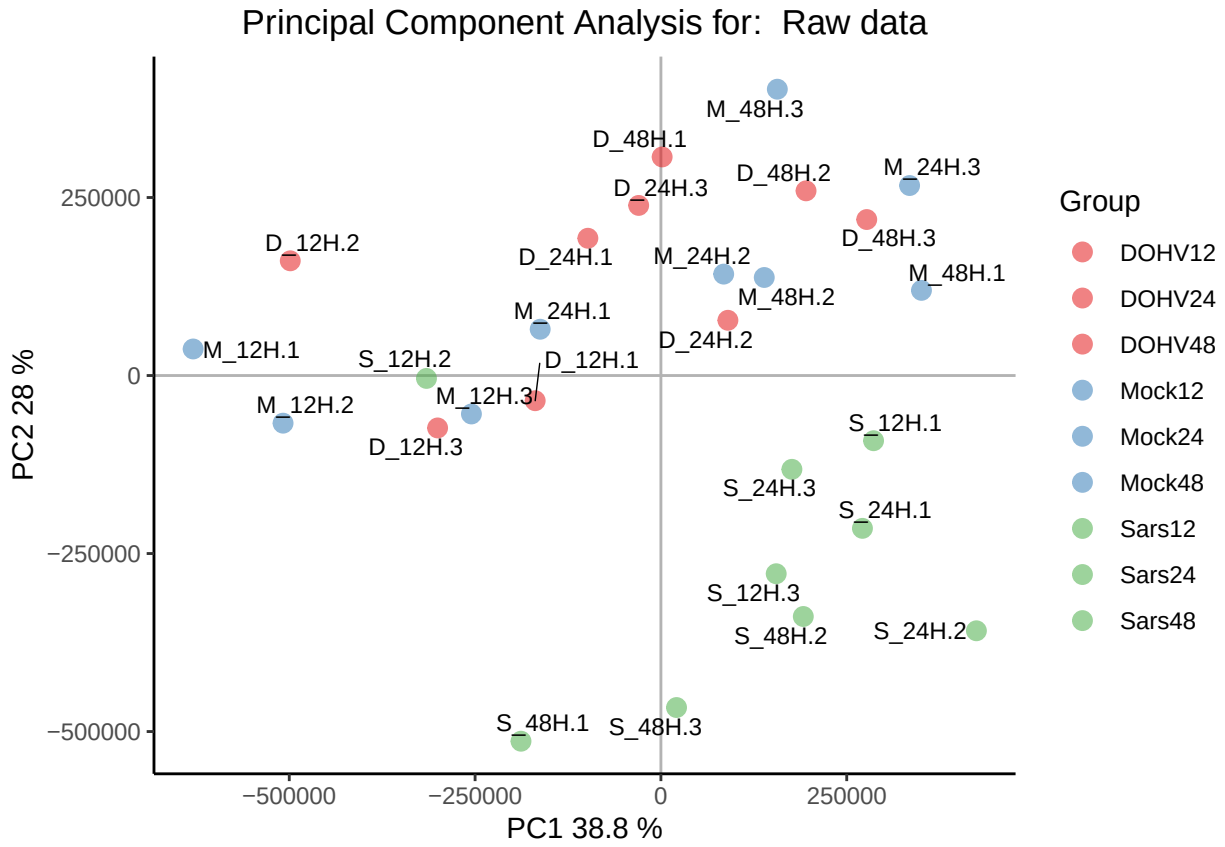
b) Quality control of the raw data.

We perform a quality control of the data with the package arrayQualityMetrics. The results can be found on the apendix. There two marks on two of the samples, but we decided to use all the samples as none of them had issues with the three metrics, and they wre used on the reference study. A deeper analysis might be performed to clarify the correctness of this data.

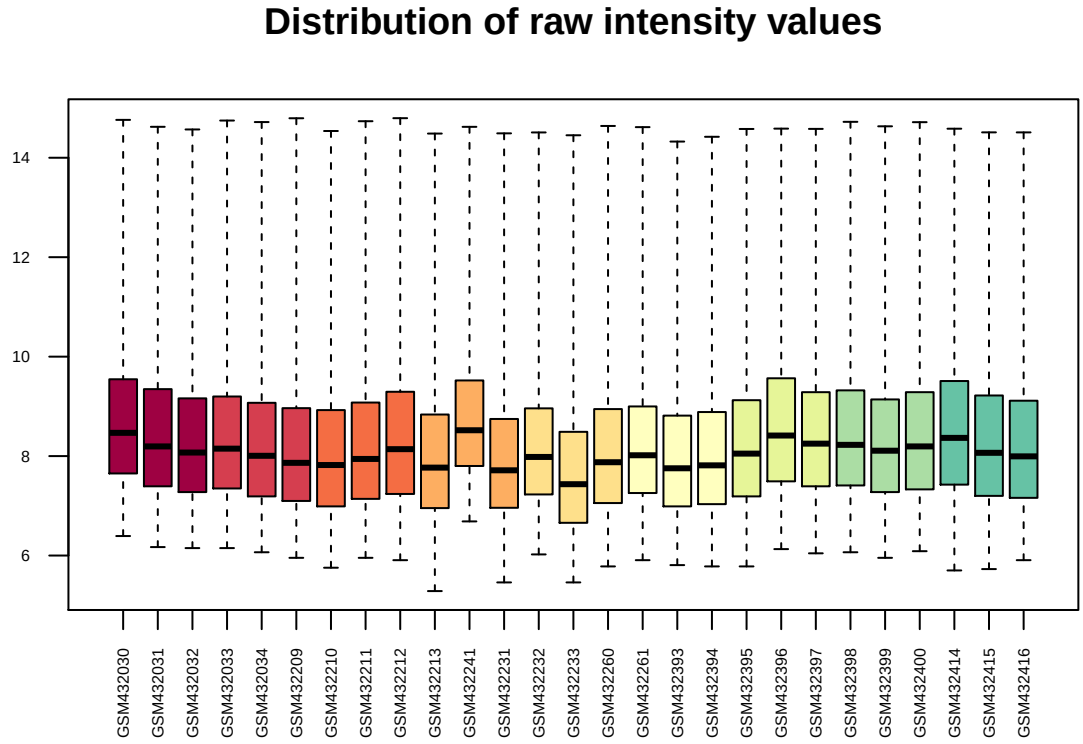
Table 1: Content of the targe file used for the analysis

FileName	Group	Day	ShortName
GSM432030	Mock12	12h	M_12H.1
GSM432031	Mock12	12h	M_12H.2
GSM432032	Mock12	12h	M_12H.3
GSM432033	Mock24	24h	M_24H.1
GSM432034	Mock24	24h	M_24H.2
GSM432209	Mock24	24h	M_24H.3
GSM432210	Mock48	48h	M_48H.1
GSM432211	Mock48	48h	M_48H.2
GSM432212	Mock48	48h	M_48H.3
GSM432213	Sars12	12h	S_12H.1
GSM432241	Sars12	12h	S_12H.2
GSM432231	Sars12	12h	S_12H.3
GSM432232	Sars24	24h	S_24H.1
GSM432233	Sars24	24h	S_24H.2
GSM432260	Sars24	24h	S_24H.3
GSM432261	Sars48	48h	S_48H.1
GSM432393	Sars48	48h	S_48H.2
GSM432394	Sars48	48h	S_48H.3
GSM432395	DOHV12	12h	D_12H.1
GSM432396	DOHV12	12h	D_12H.2
GSM432397	DOHV12	12h	D_12H.3
GSM432398	DOHV24	24h	D_24H.1
GSM432399	DOHV24	24h	D_24H.2
GSM432400	DOHV24	24h	D_24H.3
GSM432414	DOHV48	48h	D_48H.1
GSM432415	DOHV48	48h	D_48H.2
GSM432416	DOHV48	48h	D_48H.3

We also performed a Principal Components Analysis and boxplot proyection that did not show any special problems with the data.



And now we examine the intensity distribution on the arrays chcking the boxplots.

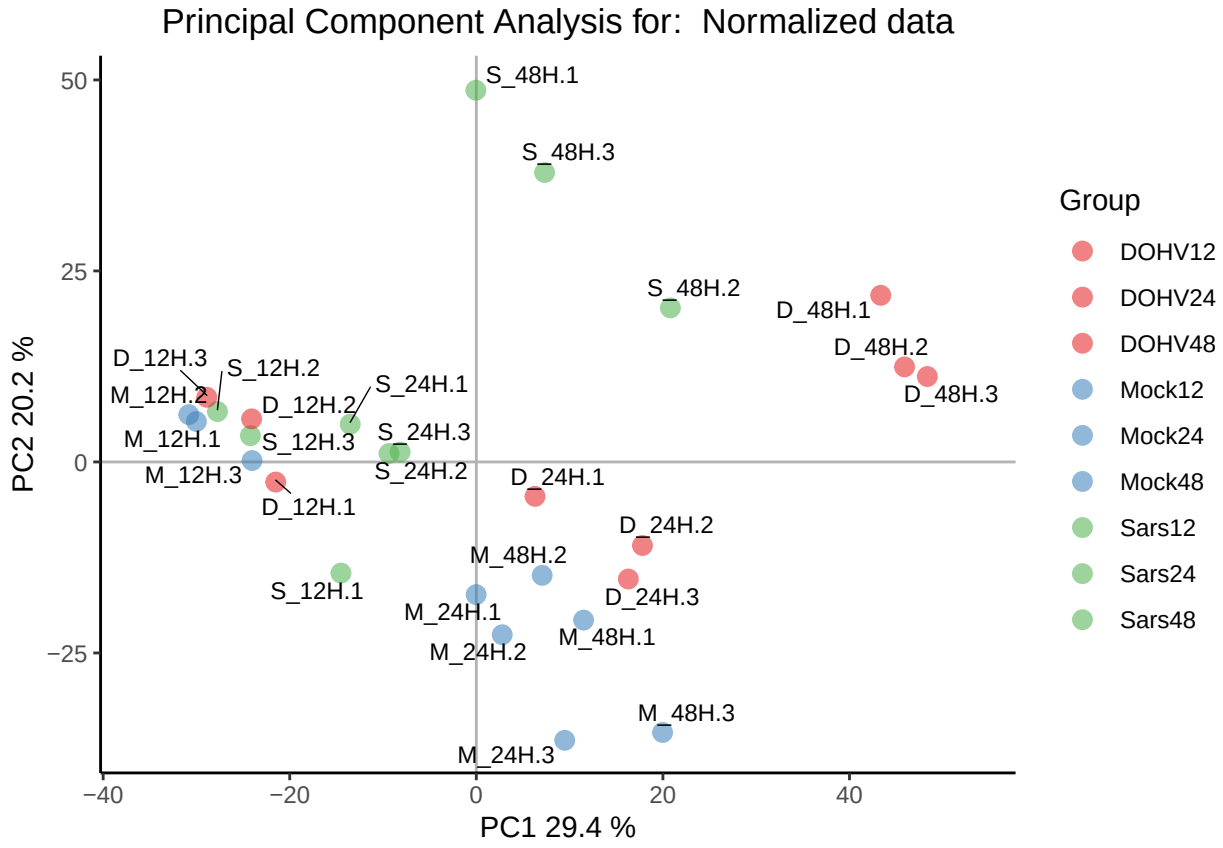


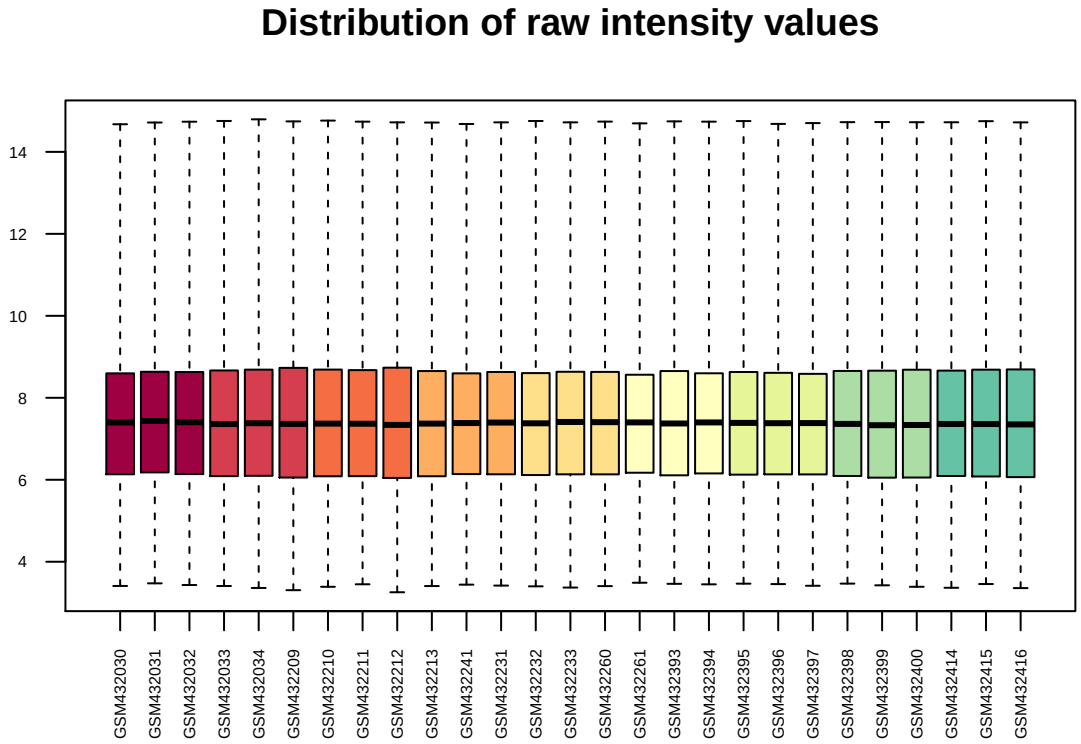
c) Data Normalization.

We use the RMA (Robust Multichip Analysis) to perform background correction, normalization and summarize the data.

d) Quality control of the normalized data.

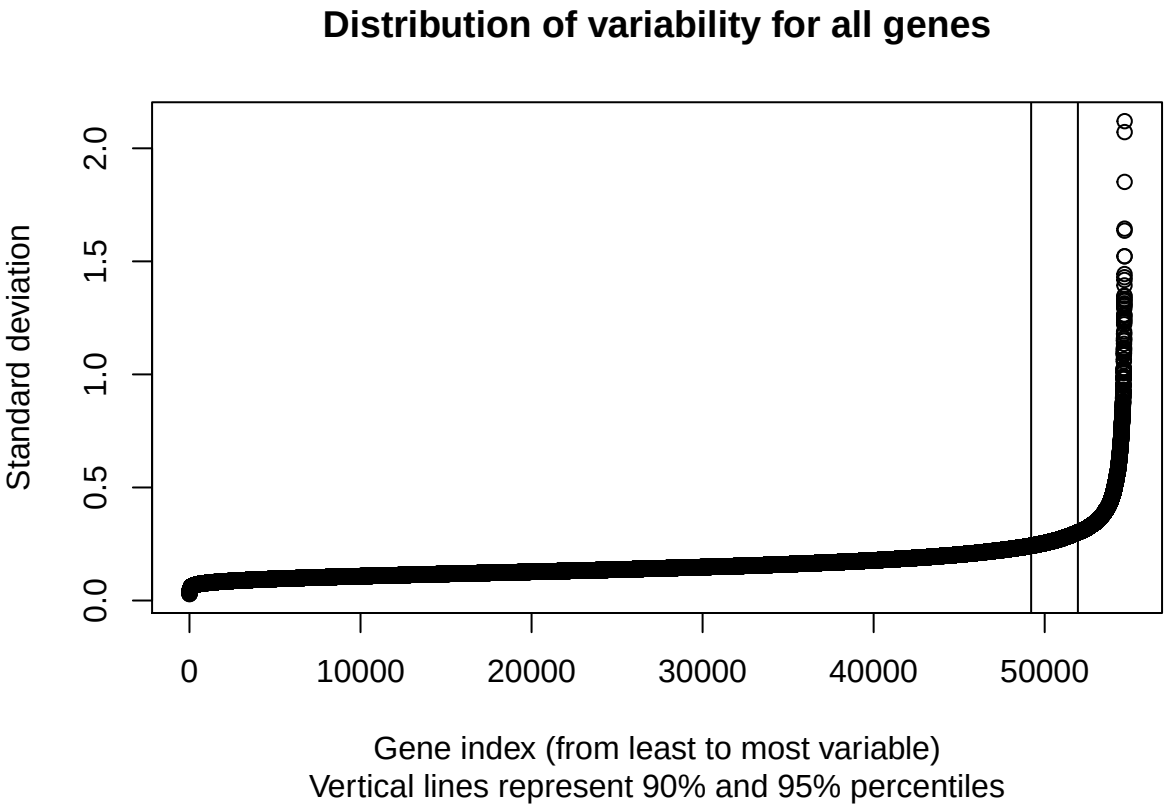
We repoeat the same analysis with the normalized data. As expected now the first component accounts for less total variability, the percentage of explained variability has decreased with respect to PCA performed on raw data. Similarly the boxplots appear with similar aspect. Which suggest the distribution of the normalized intensities is the same in all samples.





e) Identify Differentially expresed genes.

We want to identify the genes that have been differentialy expressed. First we show the standard deviations of all genes sorted from smallest to biggest values. The plot shows that the most variable genes are those with a standard deviation above 90-95% of all standard deviations.



And we use the function nsFilter from the bioconductor package genefilter to remove genes based on a variability threshold. As we have the annotation package be use it also to remove probesets which do not have a gene identifier associated.
After that we save the normalized and filtered data on the results folder.

f) Definition of the experimental setup.

We use the Linear Models for Microarrays method, as implemented in the limma package (Law et al. 2018) is used to select differential expressed genes.
The design matrix can be defined manually or from a factor variable that may have been introduced in the "targets" file with this aim created specifically for it. In this study that "Group" variable is a combination of the two experimental conditions, Infection Status and time which are jointly represented as one factor with 9 levels.

```
          DOHV12 DOHV24 DOHV48 Mock12 Mock24 Mock48 Sars12 Sars24 Sars48
GSM432030      0      0      0      1      0      0      0      0      0
GSM432031      0      0      0      1      0      0      0      0      0
GSM432032      0      0      0      1      0      0      0      0      0
GSM432033      0      0      0      0      1      0      0      0      0
GSM432034      0      0      0      0      1      0      0      0      0
GSM432209      0      0      0      0      1      0      0      0      0
GSM432210      0      0      0      0      0      1      0      0      0
GSM432211      0      0      0      0      0      1      0      0      0
GSM432212      0      0      0      0      0      1      0      0      0
GSM432213      0      0      0      0      0      0      1      0      0
GSM432241      0      0      0      0      0      0      1      0      0
GSM432231      0      0      0      0      0      0      1      0      0
GSM432232      0      0      0      0      0      0      0      1      0
GSM432233      0      0      0      0      0      0      0      1      0
GSM432260      0      0      0      0      0      0      0      1      0
GSM432261      0      0      0      0      0      0      0      0      1
GSM432393      0      0      0      0      0      0      0      0      1
GSM432394      0      0      0      0      0      0      0      0      1
GSM432395      1      0      0      0      0      0      0      0      0
GSM432396      1      0      0      0      0      0      0      0      0
GSM432397      1      0      0      0      0      0      0      0      0
GSM432398      0      1      0      0      0      0      0      0      0
GSM432399      0      1      0      0      0      0      0      0      0
GSM432400      0      1      0      0      0      0      0      0      0
GSM432414      0      0      1      0      0      0      0      0      0
GSM432415      0      0      1      0      0      0      0      0      0
GSM432416      0      0      1      0      0      0      0      0      0
attr(,"assign")
[1] 1 1 1 1 1 1 1 1 1
attr(,"contrasts")
attr(,"contrasts")$Group
[1] "contr.treatment"
```

g) Defining comparisons with the Contrasts Matrix.

In this experiment we do six comparions to compare the expresion on each time slot on the infected cells compared to the non infected cell (Mock).

```
> cont.matrix <- makeContrasts (
+   S12vsM12 =  Sars12 - Mock12,
+   S24vsM24 =  Sars24 - Mock24,
+   S48vsM48 =  Sars48 - Mock48,
```

```
+   D12vsM12 =  DOHV12 - Mock12,
+   D24vsM24 =  DOHV24 - Mock24,
+   D48vsM48 =  DOHV48 - Mock48,
+   levels = designMat)
> print(cont.matrix)
```

```
        Contrasts
Levels   S12vsM12 S24vsM24 S48vsM48 D12vsM12 D24vsM24 D48vsM48
  DOHV12        0        0        0        1        0        0
  DOHV24        0        0        0        0        1        0
  DOHV48        0        0        0        0        0        1
  Mock12       -1        0        0       -1        0        0
  Mock24        0       -1        0        0       -1        0
  Mock48        0        0       -1        0        0       -1
  Sars12        1        0        0        0        0        0
  Sars24        0        1        0        0        0        0
  Sars48        0        0        1        0        0        0
```

h) Model estimation and gene selection.

We proceed to estimate the model, estimate the contrasts and perform the significance tests that will lead to the decision, for each gene and each comparison, if they can be considered differential expressed. The method implemented in the package extends the traditional analysis using Empirical Bayes models to combine an estimate of variability based on the entire matrix with individual estimates based on each individual values providing improved error estimates. The analysis provides the usual test statistics such as Fold-change t-moderated or adjusted p-values that are used to order the genes from more unless differential expressed. In order to control the percentage of false positives that may result from high number of contrasts made simultaneously the p-values are adjusted so that we have control over the false positive rate using the Benjamini and Hochberg method.

i) Obtaining lists of differentially expressed genes.

We create a list `cont.list` using the package `topTable` to get a list of the genes more differentially expressed for each contrast.

j) Gene Anotation.

Now we enrich the data with information from the package `hgu133plus2.db`. For each list of genes in the list of contrast we add the information about Gene Name and Symbol and EntrezID to the information already present.

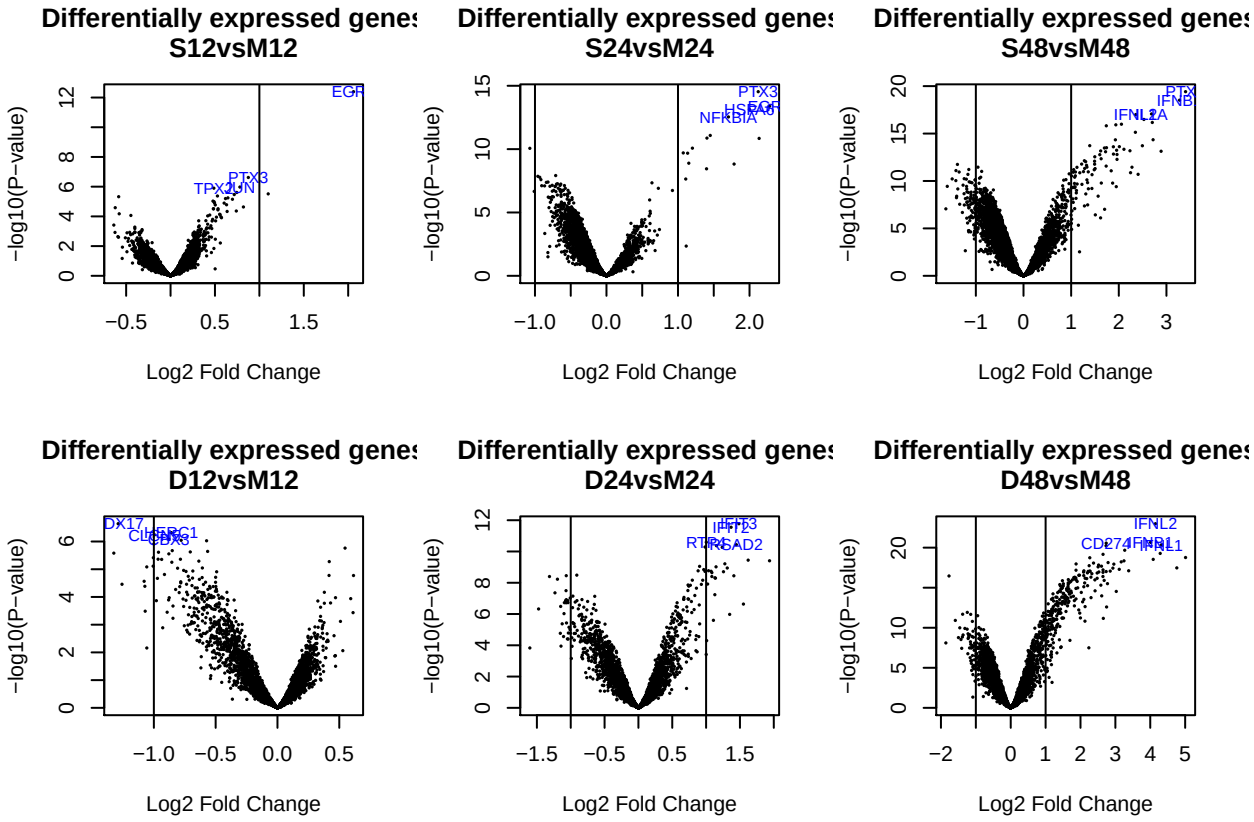
Here two examples of part of the enriched data from the contrasts.

```
[1] "Annotations added to results topTable for the comparison:  S12vsM12"
     SYMBOL                                                GENENAME
3897   EGR1                                 early growth response 1
1452   PTX3                                              pentraxin 3
626     JUN Jun proto-oncogene, AP-1 transcription factor subunit
1913   TPX2                     TPX2 microtubule nucleation factor
632  NFKBIA                                   NFKB inhibitor alpha
1598   EGR4                                 early growth response 4
[1] "Annotations added to results topTable for the comparison:  S24vsM24"
     SYMBOL                                                GENENAME
1452   PTX3                                              pentraxin 3
3897   EGR1                                 early growth response 1
2303  HSPA6          heat shock protein family A (Hsp70) member 6
632  NFKBIA                                   NFKB inhibitor alpha
626     JUN Jun proto-oncogene, AP-1 transcription factor subunit
```

1598 EGR4 early growth response 4

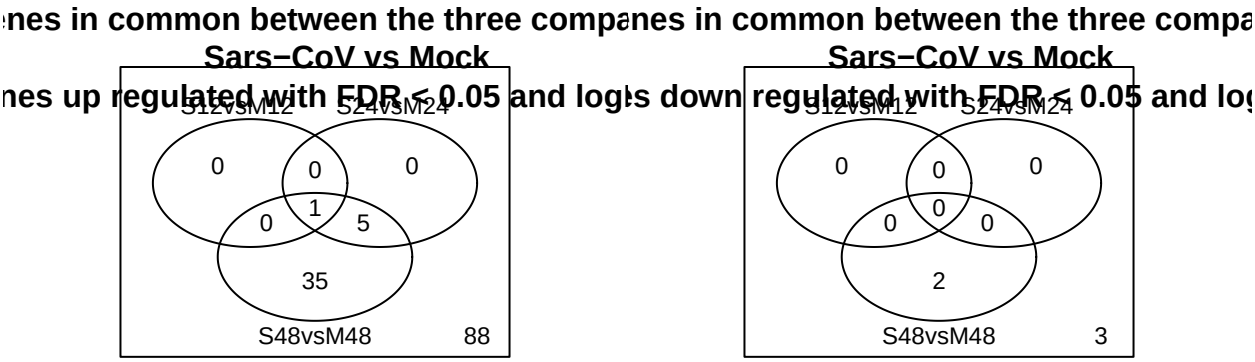
k) Visualization of differential expression.

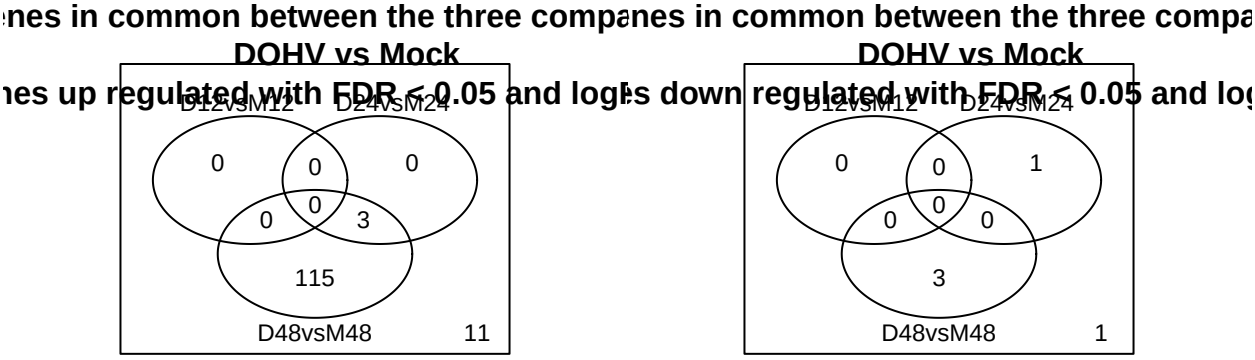
We provide a visualization of the overall differential expression using volcano-plots. These plots show if there are many or few genes with a large fold-change and significantly expressed or if this number is low. These graphs represent in the X-axis the changes of expression in logarithmic scale ("biological effect") and in the Y-axis the "minus logarithm" of the p-value or alternatively the B statistic ("Statistical effect").



l) Multiple comparisons.

Following the same criteria as the study for the experiment we are reproducing we select the genes that have been up and down regulated in the 3 time positions for each infection. We consider only genes with a folder change greater than 1.5 and $p < 0.05$.





m) Heatmaps.

To visualize the expression values of differential expressed genes we use a heatmap. We plot them doing a hierarchical clustering on genes (rows) or columns(samples) in order to find groups of genes with common patterns of variation which can eventually be associated to the different groups being compared.
We use the previosly selected genes for the heatmap. As some of the number of up regulated genes selected is very large we will use the heatmap only for the down regulated which we have considered to be more differentialy expressed ($FRD < 0.05$ y $logFC > 1.5$)

```
$rowInd
[1] 5 4 3 2 1

$colInd
 [1]  1  2  3  4  5  6  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
[26] 26 27

$call
heatmap.2(x = HMdata, Rowv = FALSE, Colv = FALSE, dendrogram = "none",
    scale = "row", col = my_palette, sepcolor = "white", sepwidth = c(0.05,
        0.05), tracecol = NULL, ColSideColors = c(rep(colorsq[1],
        9), rep(colorsq[2], 9), rep(colorsq[3], 9)), cexRow = 1,
    cexCol = 0.8, srtCol = 30, key = TRUE, keysize = 1.5, density.info = "histogram",
    main = "Differentially expressed genes \n FDR < 0.05, logFC >=1.5")

$rowMeans
   SPTBN1    DNAJC3      ACO1    NDUFS1     CMTM4
10.357489  9.529443  9.288277  7.964487 11.607288

$rowSDs
   SPTBN1    DNAJC3      ACO1    NDUFS1     CMTM4
```

```
0.7189249 0.4792412 0.7099252 0.7596154 0.5670847

$carpet
               SPTBN1       DNAJC3        ACO1     NDUFS1       CMTM4
GSM432030  0.96720986  0.039655651  0.67736284  0.8591748  0.32848042
GSM432031  0.67433036  0.016948158  0.64841597  1.1781952 -0.05981206
GSM432032  1.13364261  0.415189403  0.56200722  0.9525367  0.39833062
GSM432033  1.02308660  0.713433586  0.49968447  0.8743218  0.52418885
GSM432034  0.74748575  0.788910703  0.55751734  0.4682132  0.95184268
GSM432209  0.74056124  1.159893046  0.73419329  0.8067862  1.05320889
GSM432210  1.13804115  1.648843706  0.01058907  1.2542072  0.73479461
GSM432211  0.19275635  1.290654422  0.18038578  0.9966216  0.25300124
GSM432212  1.45442037  2.508663055  0.16132032  1.8432465  0.82029644
GSM432213  0.64779064  0.752575998  0.72021072  0.4562545  0.97537329
GSM432241  0.33285916  0.271779506  0.53771874  1.0124665  0.75665563
GSM432231  0.47075019 -0.483372413  0.75283434  0.5891471  0.45260288
GSM432232 -1.17385421 -0.697811385  0.44613081  0.1300388  0.87189188
GSM432233  0.74678644  0.242290770  0.37631022  0.8939570  1.07356943
GSM432260 -0.47666450 -0.522662040  0.54975014 -0.8089440  0.18857935
GSM432261 -1.97656571 -1.350105800 -1.17447970 -0.4825459 -0.86157692
GSM432393 -0.41184969 -1.576619148 -0.87755424 -1.1741187 -0.94418618
GSM432394  0.07852759 -1.639264019 -0.91248353 -0.6852609 -1.26118505
GSM432395 -0.09189950 -0.009848262  0.68052679 -0.5730377  0.19830576
GSM432396 -0.81745606 -0.573478694  0.75136446 -0.7680951  0.07268336
GSM432397 -0.72630810 -0.932983006  0.64000100 -0.8974689  0.22281162
GSM432398 -2.66488402 -1.174626713  0.38303432 -0.8238177  0.15975125
GSM432399 -0.92371158 -0.775411797 -0.12313472 -1.6213020 -0.35088504
GSM432400 -0.59543876 -0.343418882  0.34259096 -1.2307694  0.03492404
GSM432414 -0.45196239 -0.558873391 -2.45004223 -0.7596606 -2.75649664
GSM432415 -0.72843597  0.221201849 -2.37902277 -1.1652085 -1.74926533
GSM432416  0.69078217  0.568435699 -2.29523160 -1.3249375 -2.08788502

$rowDendrogram
'dendrogram' with 2 branches and 5 members total, at height 1.414214

$colDendrogram
'dendrogram' with 2 branches and 27 members total, at height 1.414214

$breaks
  [1] -2.756496643 -2.738058538 -2.719620434 -2.701182329 -2.682744224
  [6] -2.664306120 -2.645868015 -2.627429910 -2.608991806 -2.590553701
 [11] -2.572115596 -2.553677492 -2.535239387 -2.516801283 -2.498363178
 [16] -2.479925073 -2.461486969 -2.443048864 -2.424610759 -2.406172655
 [21] -2.387734550 -2.369296445 -2.350858341 -2.332420236 -2.313982132
 [26] -2.295544027 -2.277105922 -2.258667818 -2.240229713 -2.221791608
 [31] -2.203353504 -2.184915399 -2.166477294 -2.148039190 -2.129601085
 [36] -2.111162981 -2.092724876 -2.074286771 -2.055848667 -2.037410562
 [41] -2.018972457 -2.000534353 -1.982096248 -1.963658144 -1.945220039
 [46] -1.926781934 -1.908343830 -1.889905725 -1.871467620 -1.853029516
 [51] -1.834591411 -1.816153306 -1.797715202 -1.779277097 -1.760838993
 [56] -1.742400888 -1.723962783 -1.705524679 -1.687086574 -1.668648469
 [61] -1.650210365 -1.631772260 -1.613334155 -1.594896051 -1.576457946
 [66] -1.558019842 -1.539581737 -1.521143632 -1.502705528 -1.484267423
 [71] -1.465829318 -1.447391214 -1.428953109 -1.410515005 -1.392076900
```

```
 [76] -1.373638795 -1.355200691 -1.336762586 -1.318324481 -1.299886377
 [81] -1.281448272 -1.263010167 -1.244572063 -1.226133958 -1.207695854
 [86] -1.189257749 -1.170819644 -1.152381540 -1.133943435 -1.115505330
 [91] -1.097067226 -1.078629121 -1.060191016 -1.041752912 -1.023314807
 [96] -1.004876703 -0.986438598 -0.968000493 -0.949562389 -0.931124284
[101] -0.912686179 -0.894248075 -0.875809970 -0.857371865 -0.838933761
[106] -0.820495656 -0.802057552 -0.783619447 -0.765181342 -0.746743238
[111] -0.728305133 -0.709867028 -0.691428924 -0.672990819 -0.654552715
[116] -0.636114610 -0.617676505 -0.599238401 -0.580800296 -0.562362191
[121] -0.543924087 -0.525485982 -0.507047877 -0.488609773 -0.470171668
[126] -0.451733564 -0.433295459 -0.414857354 -0.396419250 -0.377981145
[131] -0.359543040 -0.341104936 -0.322666831 -0.304228726 -0.285790622
[136] -0.267352517 -0.248914413 -0.230476308 -0.212038203 -0.193600099
[141] -0.175161994 -0.156723889 -0.138285785 -0.119847680 -0.101409575
[146] -0.082971471 -0.064533366 -0.046095262 -0.027657157 -0.009219052
[151]  0.009219052  0.027657157  0.046095262  0.064533366  0.082971471
[156]  0.101409575  0.119847680  0.138285785  0.156723889  0.175161994
[161]  0.193600099  0.212038203  0.230476308  0.248914413  0.267352517
[166]  0.285790622  0.304228726  0.322666831  0.341104936  0.359543040
[171]  0.377981145  0.396419250  0.414857354  0.433295459  0.451733564
[176]  0.470171668  0.488609773  0.507047877  0.525485982  0.543924087
[181]  0.562362191  0.580800296  0.599238401  0.617676505  0.636114610
[186]  0.654552715  0.672990819  0.691428924  0.709867028  0.728305133
[191]  0.746743238  0.765181342  0.783619447  0.802057552  0.820495656
[196]  0.838933761  0.857371865  0.875809970  0.894248075  0.912686179
[201]  0.931124284  0.949562389  0.968000493  0.986438598  1.004876703
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244  1.723962783  1.742400888 #AB6E8E
245  1.742400888  1.760838993 #AA6E8E
246  1.760838993  1.779277097 #AA6D8F
247  1.779277097  1.797715202 #A96C8F
248  1.797715202  1.816153306 #A96C8F
249  1.816153306  1.834591411 #A96B90
250  1.834591411  1.853029516 #A86B90
251  1.853029516  1.871467620 #A86A90
252  1.871467620  1.889905725 #A86991
253  1.889905725  1.908343830 #A76991
254  1.908343830  1.926781934 #A76892
255  1.926781934  1.945220039 #A76892
256  1.945220039  1.963658144 #A66792
257  1.963658144  1.982096248 #A66693
258  1.982096248  2.000534353 #A66693
259  2.000534353  2.018972457 #A56593
260  2.018972457  2.037410562 #A56594
261  2.037410562  2.055848667 #A56494
262  2.055848667  2.074286771 #A46395
263  2.074286771  2.092724876 #A46395
264  2.092724876  2.111162981 #A46295
265  2.111162981  2.129601085 #A36296
266  2.129601085  2.148039190 #A36196
267  2.148039190  2.166477294 #A36196
268  2.166477294  2.184915399 #A26097
269  2.184915399  2.203353504 #A25F97
270  2.203353504  2.221791608 #A25F98
271  2.221791608  2.240229713 #A15E98
272  2.240229713  2.258667818 #A15E98
273  2.258667818  2.277105922 #A05D99
274  2.277105922  2.295544027 #A05C99
275  2.295544027  2.313982132 #A05C99
276  2.313982132  2.332420236 #9F5B9A
277  2.332420236  2.350858341 #9F5B9A
278  2.350858341  2.369296445 #9F5A9B
279  2.369296445  2.387734550 #9E599B
280  2.387734550  2.406172655 #9E599B
281  2.406172655  2.424610759 #9E589C
282  2.424610759  2.443048864 #9D589C
```

```
283  2.443048864  2.461486969 #9D579C
284  2.461486969  2.479925073 #9D569D
285  2.479925073  2.498363178 #9C569D
286  2.498363178  2.516801283 #9C559E
287  2.516801283  2.535239387 #9C559E
288  2.535239387  2.553677492 #9B549E
289  2.553677492  2.572115596 #9B539F
290  2.572115596  2.590553701 #9B539F
291  2.590553701  2.608991806 #9A529F
292  2.608991806  2.627429910 #9A52A0
293  2.627429910  2.645868015 #9A51A0
294  2.645868015  2.664306120 #9950A1
295  2.664306120  2.682744224 #9950A1
296  2.682744224  2.701182329 #994FA1
297  2.701182329  2.719620434 #984FA2
298  2.719620434  2.738058538 #984EA2
299  2.738058538  2.756496643 #984EA3

$layout
$layout$lmat
     [,1] [,2]
[1,]    5    4
[2,]    0    1
[3,]    3    2

$layout$lhei
[1] 1.5 0.2 4.0

$layout$lwid
[1] 1.5 4.0
```

```
pdf
  2
```

n) Biological significance of results.

To obtain some insights about the biological significance of the results we use the pipelines described on the clusterProfiler package (Yu n.d.).
Here we describe some of the enrichment options for the clusterProfiler package:
* enrichDO: Disiease Association
* enrichNCG: A manually curated repository of cancer genes from the Network of Cancer Gene (NCG) ("Network of Cancer Genes Home" n.d.)
* enrichDGN: gene-disease associations from DisGeNET. ("DisGeNET - a Database of Gene-Disease Associations" n.d.)
* groupGO, enrichGO and gseGO: GO analyses for organisms that have an OrgDb object available.

Once we have prepared and enriched the data following their instructions, we perform a series of example analysis.
The most interesting is with `browseKEGG` to be able to whatch the relevant overexpressed genes in a certain pathway.

```
S12vsM12 S24vsM24 S48vsM48 D12vsM12 D24vsM24 D48vsM48
       1        6       41        0        3      118
```

```
> kk <- enrichKEGG(gene         = gene,
+                  organism     = 'hsa',
+                  pvalueCutoff = 0.05)
```

```
>
> head(kk)
```

```
               ID                          Description GeneRatio  BgRatio
hsa05164 hsa05164                          Influenza A     23/70 170/8039
hsa04621 hsa04621    NOD-like receptor signaling pathway     16/70 181/8039
hsa05169 hsa05169          Epstein-Barr virus infection     15/70 201/8039
hsa05162 hsa05162                               Measles     13/70 138/8039
hsa04060 hsa04060 Cytokine-cytokine receptor interaction     17/70 294/8039
hsa05168 hsa05168       Herpes simplex virus 1 infection     21/70 491/8039
               pvalue     p.adjust       qvalue
hsa05164 5.105681e-22 5.718363e-20 3.117153e-20
hsa04621 1.927982e-12 1.079670e-10 5.885420e-11
hsa05169 1.191519e-10 3.667053e-09 1.998957e-09
hsa05162 1.309662e-10 3.667053e-09 1.998957e-09
hsa04060 3.229812e-10 7.234779e-09 3.943771e-09
hsa05168 4.869733e-10 9.043848e-09 4.929917e-09

hsa05164 3627/3456/6352/91543/23586/3569/64135/8743/834/4939/7098/6772/3122/4938/5371/3592/3113/3665/357
hsa04621                                    3456/115361/6352/3569/834/4939/330/2633/6772/4938/3665/35
hsa05169                                       3627/3456/23586/3569/4939/6890/6772/3122/6891/4938/3
hsa05162                                                3456/23586/3569/64135/4939/6772/4938/3592/3
hsa04060                          6373/3627/282618/282616/3456/6352/10673/3569/6376/8743/51554/6364/359
hsa05168                3456/6352/23586/3569/64135/4939/330/7098/684/6890/6772/3122/6891/4938/5371/3592/3
         Count
hsa05164    23
hsa04621    16
hsa05169    15
hsa05162    13
hsa04060    17
hsa05168    21
```

```
> # browseKEGG(kk, 'hsa05164')
```

4 Results

The

5 Discusion

`limma` offers a straightforward approach to gene expression analysis. Another great surprise was the large number of possibilities that the `clusterProfiler` package includes to perform deeper pathway analysis starting from the results of the experiment.

6 Apendix

6.1 R packages list

knitr
https://yihui.org/knitr/
BiocManager
https://cran.r-project.org/web/packages/BiocManager/vignettes/BiocManager.html

Biobase
https://www.bioconductor.org/packages/release/bioc/html/Biobase.html

kableExtra
https://cran.r-project.org/web/packages/kableExtra/vignettes/awesome_table_in_html.html

oligo
https://www.bioconductor.org/packages/release/bioc/html/oligo.html

pvca
https://www.bioconductor.org/packages/release/bioc/html/pvca.html

ggrepel
https://cran.r-project.org/web/packages/ggrepel/vignettes/ggrepel.html

colorspace
https://cran.r-project.org/web/packages/colorspace/vignettes/colorspace.html

gplots
https://cran.r-project.org/web/packages/ggplot2/index.html

ggupset
https://github.com/const-ae/ggupset

magrittr
https://cran.r-project.org/web/packages/magrittr/vignettes/magrittr.html

clusterProfiler
https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html

DOSE
https://www.bioconductor.org/packages/release/bioc/html/DOSE.html

RColorBrewer
https://www.rdocumentation.org/packages/RColorBrewer/versions/1.1-2/topics/RColorBrewer

ggplot2
https://ggplot2.tidyverse.org/

hgu133plus2.db
http://bioconductor.org/packages/release/data/annotation/html/hgu133plus2.db.html

arrayQualityMetrics
https://www.bioconductor.org/packages/release/bioc/html/arrayQualityMetrics.html

limma
https://www.bioconductor.org/packages/release/bioc/html/limma.html

genefilter
https://bioconductor.org/packages/release/bioc/html/genefilter.html

annotate
https://www.bioconductor.org/packages/release/bioc/html/annotate.html

enrichplot
https://bioconductor.org/packages/release/bioc/html/enrichplot.html

	array	sampleNames	*1	*2	*3	Group	Day	ShortName
☐	1	GSM432030			x	Mock12	12h	M_12H.1
☐	2	GSM432031			x	Mock12	12h	M_12H.2
☐	3	GSM432032				Mock12	12h	M_12H.3
☐	4	GSM432033				Mock24	24h	M_24H.1
☐	5	GSM432034				Mock24	24h	M_24H.2
☐	6	GSM432209				Mock24	24h	M_24H.3
☐	7	GSM432210				Mock48	48h	M_48H.1
☐	8	GSM432211				Mock48	48h	M_48H.2
☐	9	GSM432212			x	Mock48	48h	M_48H.3
☐	10	GSM432213			x	SarsCoV12	12h	S_12H.1
☐	11	GSM432241		x	x	SarsCoV12	12h	S_12H.2
☐	12	GSM432231			x	SarsCoV12	12h	S_12H.3
☐	13	GSM432232			x	SarsCoV24	24h	S_24H.1
☐	14	GSM432233		x	x	SarsCoV24	24h	S_24H.2
☐	15	GSM432260				SarsCoV24	24h	S_24H.3
☐	16	GSM432261				SarsCoV48	48h	S_48H.1
☐	17	GSM432393			x	SarsCoV48	48h	S_48H.2
☐	18	GSM432394			x	SarsCoV48	48h	S_48H.3
☐	19	GSM432395				DOHV12	12h	D_12H.1
☐	20	GSM432396			x	DOHV12	12h	D_12H.2
☐	21	GSM432397				DOHV12	12h	D_12H.3
☐	22	GSM432398			x	DOHV24	24h	D_24H.1
☐	23	GSM432399				DOHV24	24h	D_24H.2
☐	24	GSM432400				DOHV24	24h	D_24H.3
☐	25	GSM432414			x	DOHV48	48h	D_48H.1
☐	26	GSM432415				DOHV48	48h	D_48H.2
☐	27	GSM432416				DOHV48	48h	D_48H.3

Figure 1: Raw data quality control

	array	sampleNames	*1	*2	*3	Group	Day	ShortName
☐	1	GSM432030				Mock12	12h	M_12H.1
☐	2	GSM432031				Mock12	12h	M_12H.2
☐	3	GSM432032				Mock12	12h	M_12H.3
☐	4	GSM432033				Mock24	24h	M_24H.1
☐	5	GSM432034				Mock24	24h	M_24H.2
☐	6	GSM432209				Mock24	24h	M_24H.3
☐	7	GSM432210				Mock48	48h	M_48H.1
☐	8	GSM432211				Mock48	48h	M_48H.2
☐	9	GSM432212				Mock48	48h	M_48H.3
☐	10	GSM432213				SarsCoV12	12h	S_12H.1
☐	11	GSM432241				SarsCoV12	12h	S_12H.2
☐	12	GSM432231				SarsCoV12	12h	S_12H.3
☐	13	GSM432232				SarsCoV24	24h	S_24H.1
☐	14	GSM432233				SarsCoV24	24h	S_24H.2
☐	15	GSM432260				SarsCoV24	24h	S_24H.3
☐	16	GSM432261	x			SarsCoV48	48h	S_48H.1
☐	17	GSM432393				SarsCoV48	48h	S_48H.2
☐	18	GSM432394				SarsCoV48	48h	S_48H.3
☐	19	GSM432395				DOHV12	12h	D_12H.1
☐	20	GSM432396				DOHV12	12h	D_12H.2
☐	21	GSM432397				DOHV12	12h	D_12H.3
☐	22	GSM432398				DOHV24	24h	D_24H.1
☐	23	GSM432399				DOHV24	24h	D_24H.2
☐	24	GSM432400				DOHV24	24h	D_24H.3
☐	25	GSM432414				DOHV48	48h	D_48H.1
☐	26	GSM432415				DOHV48	48h	D_48H.2
☐	27	GSM432416				DOHV48	48h	D_48H.3

Figure 2: Normalized data quality control

6.2 Raw data quality control

6.3 Normalized data quality control

References

"DisGeNET - a Database of Gene-Disease Associations." n.d. Accessed May 3, 2020. https://www.disgenet.org/.

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Yoshikawa, Tomoki, Terence E. Hill, Naoko Yoshikawa, Vsevolod L. Popov, Cristi L. Galindo, Harold R. Garner, C. J. Peters, and Chien-Te (Kent) Tseng. 2010. "Dynamic Innate Immune Responses of Human Bronchial Epithelial Cells to Severe Acute Respiratory Syndrome-Associated Coronavirus Infection." *PLoS One* 5 (1). https://doi.org/10.1371/journal.pone.0008729.

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