Using My R for fun and profit

Ashton Trey Belew abelew@gmail.com

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Myr: Stupid R tricks.

The following block shows how I handle autloading requisite libraries for my code. This makes it easier for me to download/install the R requirements on a new computer, something which I have found myself needing to do more than I would have guessed.

```
## This block serves to load requisite libraries and set some options.
library("myr")
## To set up an initial vignette, use the following line:
## devtools::use_vignette("myr")
autoloads()
```

```
## [1] "Loading biomaRt"
## [1] "Loading BSgenome"
## [1] "Loading BSgenome.Lmajor.friedlin"
## [1] "Loading Cairo"
## [1] "Loading cbcbSEQ"
## [1] "Loading clusterProfiler"
## [1] "Loading data.table"
## [1] "Loading DESeq2"
## [1] "Loading DESeq"
## [1] "Loading devtools"
## [1] "Loading directlabels"
## [1] "Loading DOSE"
## [1] "Loading edgeR"
## [1] "Loading genomeIntervals"
## [1] "Loading ggplot2"
## [1] "Loading GO.db"
## [1] "Loading googleVis"
## [1] "Loading goseq"
## [1] "Loading gplots"
## [1] "Loading gtools"
## [1] "Loading gridExtra"
## [1] "Loading hash"
## [1] "Loading KEGGREST"
## [1] "Loading knitcitations"
## [1] "Loading knitr"
## [1] "Loading knitrBootstrap"
## [1] "Loading methods"
## [1] "Loading motifRG"
## [1] "Loading multtest"
## [1] "Loading pathview"
## [1] "Loading plyr"
## [1] "Loading qvalue"
## [1] "Loading RamiGO"
## [1] "Loading RColorBrewer"
```

```
## [1] "Loading reshape"
## [1] "Loading Rgraphviz"
## [1] "Loading rjson"
## [1] "Loading rmarkdown"
## [1] "Loading robust"
## [1] "Loading roxygen2"
## [1] "Loading Rsamtools"
## [1] "Loading rtracklayer"
## [1] "Loading scales"
## [1] "Loading seqinr"
## [1] "Loading sva"
## [1] "Loading testthat"
## [1] "Loading topGO"
##
## groupGOTerms:    GOBPTerm, GOMFTerm, GOCCTerm environments built.
## [1] "Loading XLConnect"
## [1] "Loading xtable"
```

```
opts_knit$set(progress=TRUE, verbose=TRUE, stop_on_error=FALSE, error=TRUE, fig.width=800/192, fig.heigh
options(java.parameters="-Xmx8g")  ## used for xlconnect -- damn 4g wasn't enough
theme_set(theme_bw(base_size=10))
set.seed(1)
```

Rendering the vignette

The following block has a few lines I use to load data, save it, and render pdf/html reports. I do this under the veritable editor, 'emacs,' with the key combination "Control-c, Control-n" for each line I want to evaluate in R, or "Control-c, Control-c" for a paragraph.

```
load("RData")
rm(list=ls())
save(list=ls(all=TRUE), file="RData")
render("myr.Rmd", output_format="pdf_document")
render("myr.Rmd", output_format="html_document")
```

Tasks that Myr helps me perform

This code was written to speed up and simplify a few specific tasks:

- Reading RNA sequencing count tables (in R/count_tables.R)
- Normalization of data (R/normalization.R)
- Graphing metrics of data to check and evaludate batch effects (R/plots.R)
- Performing contrasts of the data using voom/limma (R/misc_functions.R)
- Plotting RNA abundances by condition/batch (R/plots.R)
- Simplifying ontology/KEGG searches (R/ontology.R)

The following paragraphs will attempt to show how I use it.

Annotation information

Every RNA sequencing experiment I have played with has required a different handling of the genome's annotation. Most, but not all, have kept the data of interest in a gff file. Here is an example of how I process one of those files and make a data frame of genes as well as tooltips, which will be used for googleVis graphs later. In every experiment I have played with, I make a 'reference' directory into which I copy the current annotation data, this way I have a consistent and known version of the annotation. In the example below, this is the TriTrypDB version 8.1 of the T. cruzi genome.

```
tcruzi_annotations = import.gff3("reference/gff/clbrener_8.1_complete.gff.gz")
annotation_info = as.data.frame(tcruzi_annotations)

genes = annotation_info[annotation_info$type=="gene",]
gene_annotations = genes
rownames(genes) = genes$Name
tooltip_data = genes
tooltip_data = tooltip_data[,c(11,12)]
tooltip_data$tooltip = paste(tooltip_data$Name, tooltip_data$description, sep=": ")
tooltip_data$tooltip = gsub("\\+", " ", tooltip_data$tooltip)
rownames(tooltip_data) = tooltip_data$Name
tooltip_data = tooltip_data[-1]
tooltip_data = tooltip_data[-1]
colnames(tooltip_data) = c("name.tooltip")
head(tooltip_data)
```

Reading count tables

In Dr. El-Sayed's lab, there is a very specific naming convention for RNA sequencing experiments. Every sequencing run has an 'HPGL' (host pathogen genomics lab) identifier. All experiments have associated metadata, including the condition in the experiment, the batch, bioanalyzer reports, etc. When I play with data, I keep all this information in a csv file 'samples.csv' and the processed count-tables for the experiment in a specific directory: processed_data/. Therefore, I have a couple functions which automate the import of data into R in the hopes that no mistakes are made.

Here is an example from a recent experiment.

```
samples = read.csv("data/all_samples.csv")
knitr::kable(head(samples))
```

Sample.ID	Type	Stage	batch	Media	SRA	Reads.Passed	ncRNA	X..ncRNA	Remaining	Genome	X..G
HPGL0406	WT	EL	1	THY	NA	19026277	353992	1.86%	18672285	17810587	95.3
HPGL0407	WT	EL	2	THY	NA	15074073	259613	1.72%	14814460	14334043	96.7
HPGL0408	mga	EL	1	THY	NA	17112233	293752	1.72%	16818481	15769581	93.7
HPGL0409	mga	EL	2	THY	NA	18298278	339862	1.86%	17958416	16553148	92.1
HPGL0149	WT	LL	1	THY	NA	39107368	8055417	20.60%	31051951	26285560	84.6
HPGL0150	WT	LL	2	THY	NA	35429033	3705275	10.46%	31723758	30012962	94.6

Since I didn't want to copy over all my count tables, you, dear reader, will have to trust that there is a file

for each entry in the above table which corresponds to the Sample.ID. These may be organized by sample name or condition. The following code shows how I create an expressionset and fill it with the count data.

```
example_data = counts(make_exampledata(ngenes=10000, columns=24))
## create_expt() usually expects that there are a bunch of count tables
## from htseq in the directory: processed_data/count_tables/
## These may be organised in separate directories by condition(type)
## in one directory each by sample.  By default, this assumes they will be
## named sample_id.count.gz, but this may be changed with the suffix argument.
all_expt = create_expt("data/all_samples.csv", count_dataframe=example_data)
```

```
## [1] "This function needs the conditions and batches to be an explicit column in the sample sheet."
```

```
## Warning in brewer.pal(num_colors, "Dark2"): n too large, allowed maximum for palette Dark2 is 8
## Returning the palette you asked for with that many colors
```

```
## [1] "Please note that thus function assumes a specific set of columns in the sample sheet:"
## [1] "The most important ones are: Sample.ID, Stage, Type."
## [1] "Other columns it will attempt to create by itself, but if"
## [1] "batch and condition are provided, that is a nice help."
```

Examining data

Once the data is read in, the first task is always to look at it and evaluate for batch effects and thus decide what to do about them. However, different normalization methods are appropriate in different data sets, therefore I have some functions which attempt to make this easier. For this, I will make a dummy data set using limma's makeExampleData()

```
## graph_metrics() performs the following:
## runs a libsize plot, non-zero genes plot, boxplot, correlation/distance heatmaps, and pca plots
## It performs a normalization of the data (log2(quantile(cpm)) by default), and does it again
## It then uses limma's removeBatchEffect() to make a stab at removing batch effect, and does it again.

## An important thing to remember: the data from makeExampleData() is not very interesting, so the resul
## plots are also not interesting...
fun = graph_metrics(expt=all_expt)
```

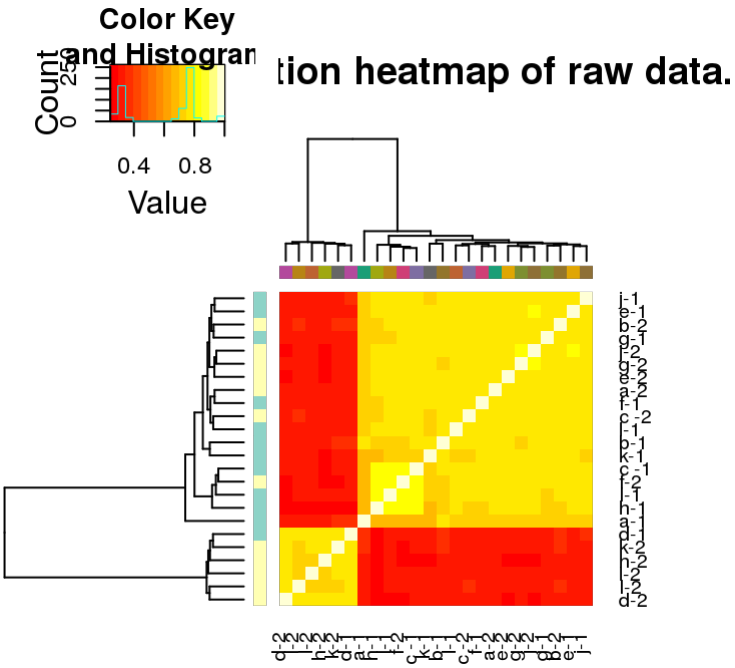
```
## [1] "Graphing number of non-zero genes with respect to CPM by library."
## [1] "Graphing library sizes."
## [1] "Adding log10"
## [1] "Graphing a raw data boxplot on log scale."
```

```
## Using id as id variables
```

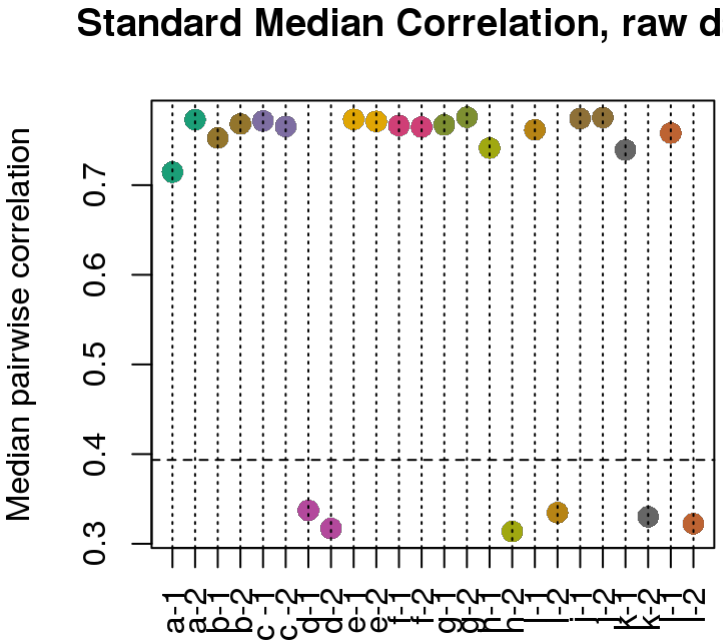
```
## [1] "Graphing a normalized boxplot."
```

```
## Using id as id variables
```

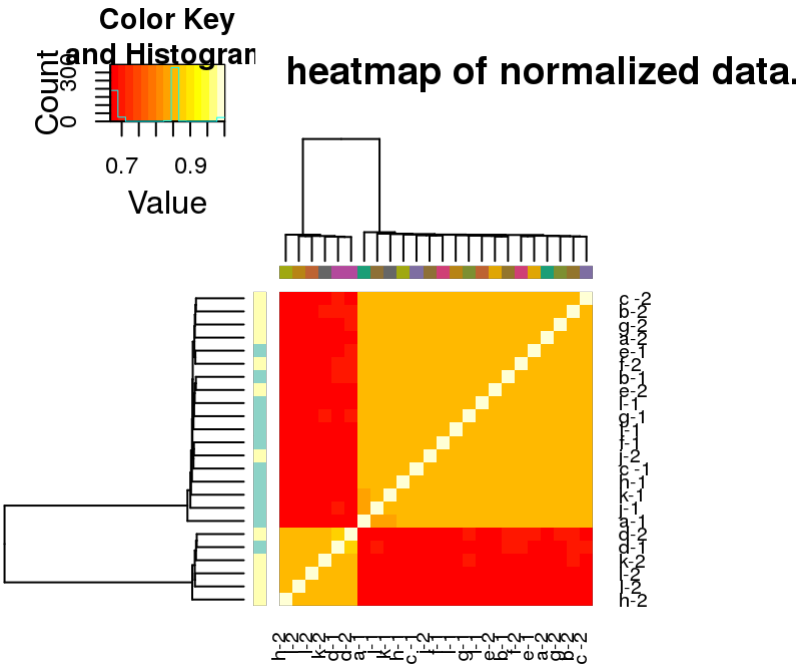
```
## [1] "Graphing a raw-data correlation heatmap."
```



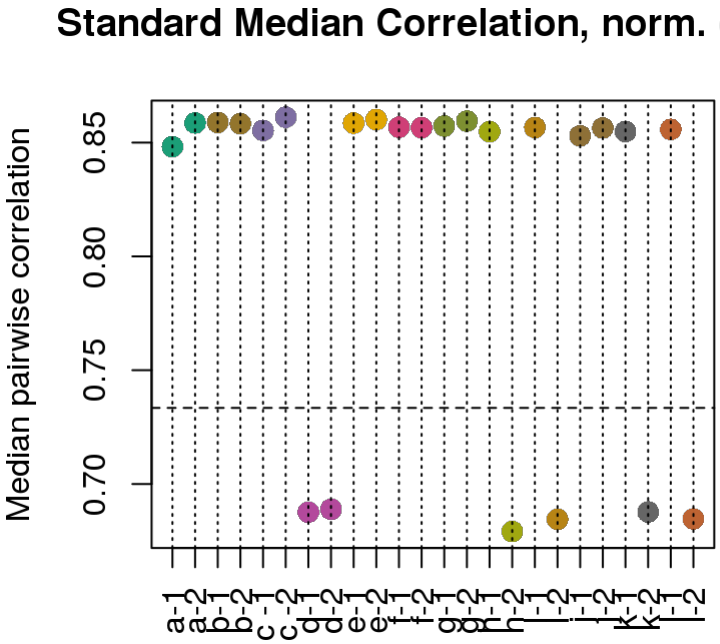

```
## [1] "Graphing a raw-data standard median correlation."
```



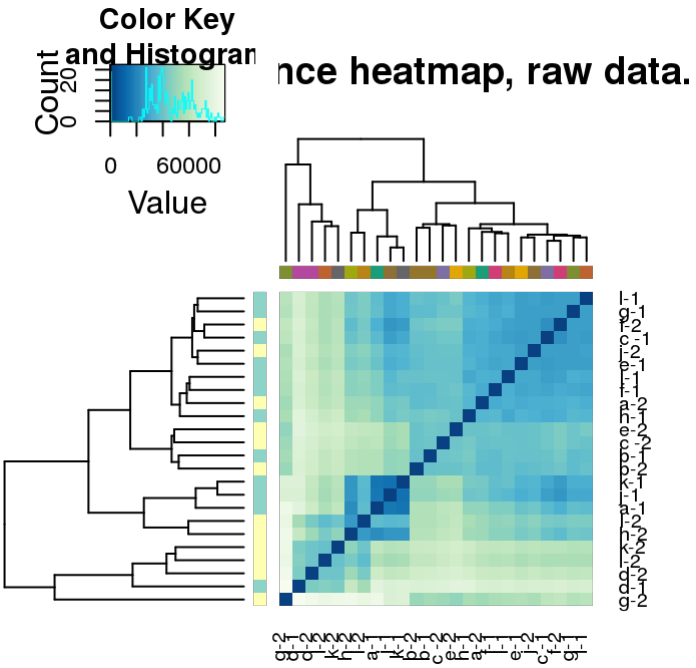

```
## [1] "Graphing a normalized correlation heatmap."
```



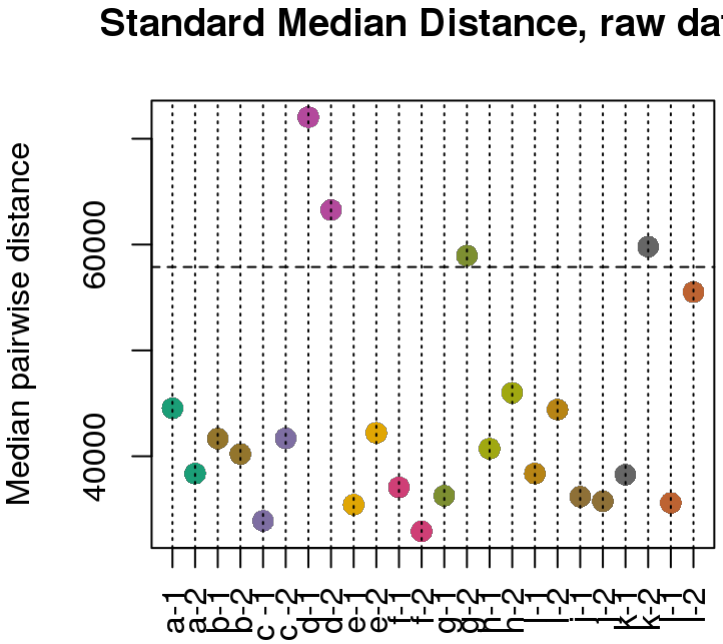

```
## [1] "Graphing a normalized standard median correlation."
```



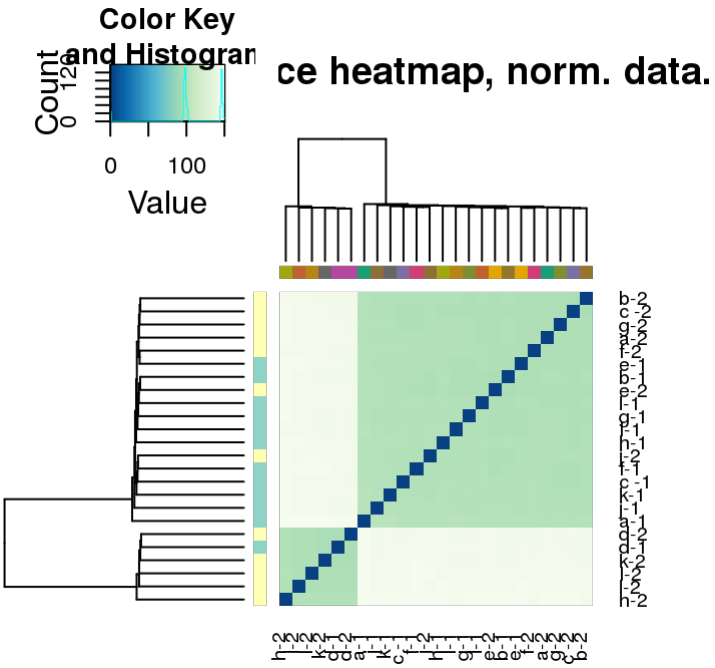

```
## [1] "Graphing a raw-data distance heatmap."
```




```
## [1] "Graphing a raw-data standard median distance."
```




```
## [1] "Graphing a normalized distance heatmap."
```

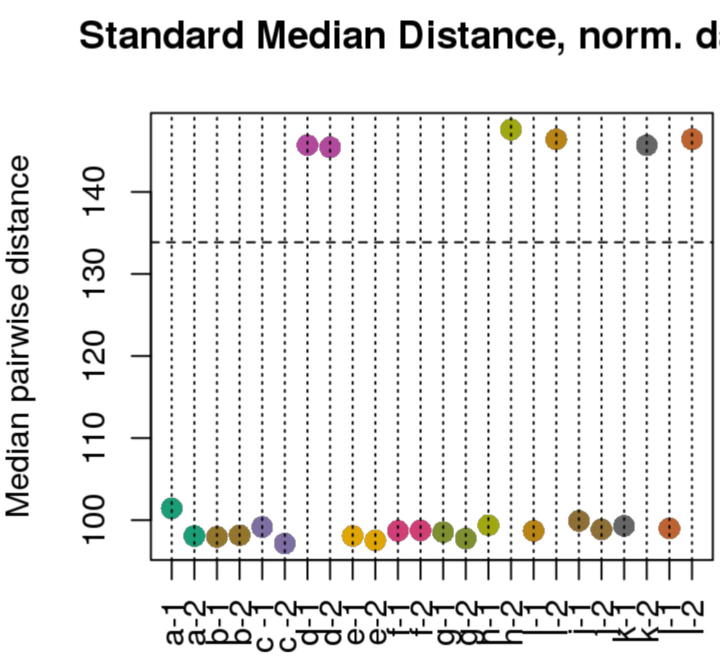


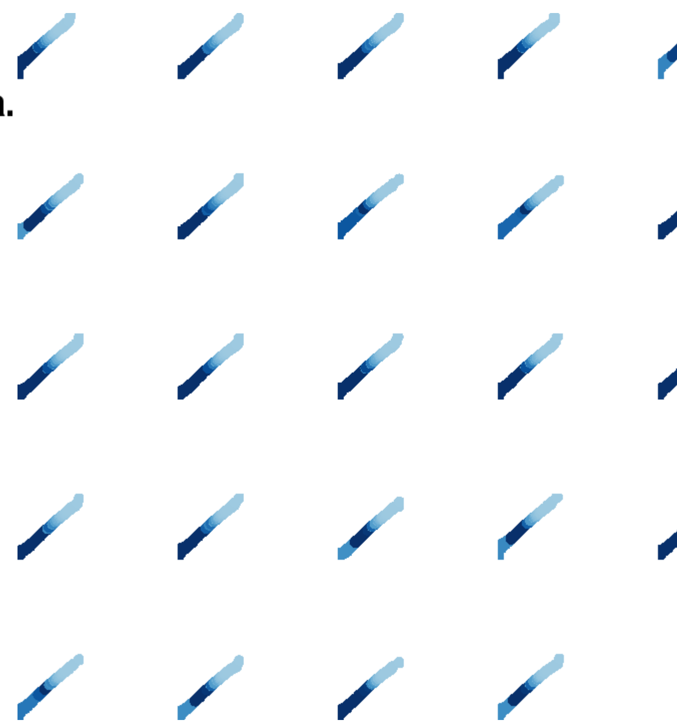

```
## [1] "Graphing a normalized standard median distance."
```

```
## [1] "Graphing a PCA plot of the raw data."
##    propVar cumPropVar cond.R2 batch.R2
## 1    38.90      38.90   65.58     2.02
## 2    22.68      61.58   48.03    12.08
## 3     4.18      65.76   55.85     2.90
## 4     3.47      69.23   60.55     1.22
## 5     3.06      72.29   13.77    16.57
## 6     2.91      75.20   51.94     0.01
## 7     2.83      78.03   58.16     2.50
## 8     2.60      80.63   40.48     3.92
## 9     2.57      83.20   29.00     2.97
## 10    2.50      85.70   31.46     6.33
## 11    1.99      87.69   55.96     4.23
## 12    1.64      89.33   50.20     9.96
## 13    1.56      90.89   52.36     0.16
## 14    1.43      92.32   33.43     0.01
## 15    1.27      93.59   45.31     0.17
## 16    1.25      94.84   50.07    16.02
## 17    1.18      96.02   46.71     3.30
## 18    1.12      97.14   60.63     0.07
## 19    1.00      98.14   40.19     0.77
## 20    0.84      98.98   54.10     6.48
## 21    0.45      99.43   53.26     8.16
## 22    0.34      99.77   54.93     0.10
## 23    0.22      99.99   48.03     0.06
## [1] "Printing a qqplot of the raw data."
```

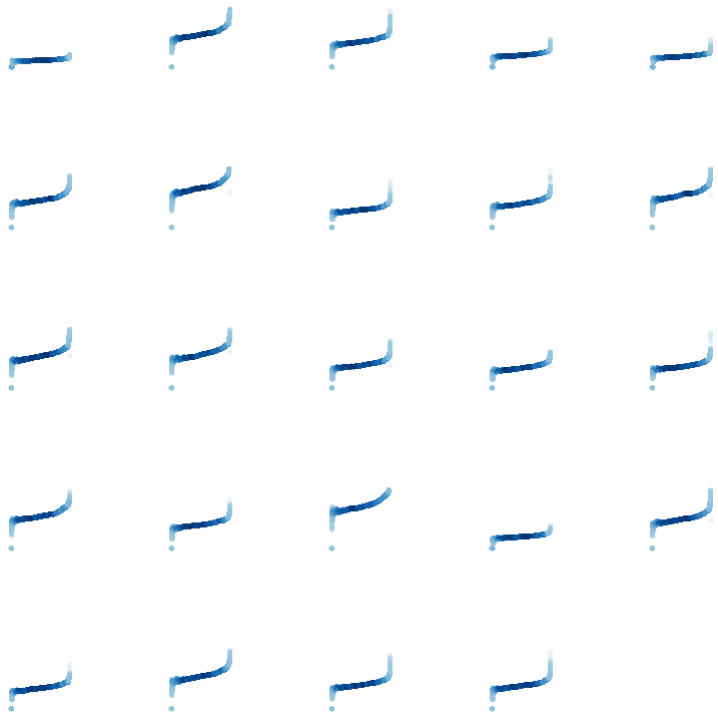
```
## Warning in KernSmooth::bkde2D(x, bandwidth = bandwidth, gridsize = nbin, :
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'
```

```
## Warning in KernSmooth::bkde2D(x, bandwidth = bandwidth, gridsize = nbin, :
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, bandwidth = bandwidth, gridsize = nbin, :
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, bandwidth = bandwidth, gridsize = nbin, :
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, bandwidth = bandwidth, gridsize = nbin, :
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, bandwidth = bandwidth, gridsize = nbin, :
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'

## Warning in KernSmooth::bkde2D(x, bandwidth = bandwidth, gridsize = nbin, :
## Binning grid too coarse for current (small) bandwidth: consider increasing
## 'gridsize'
```

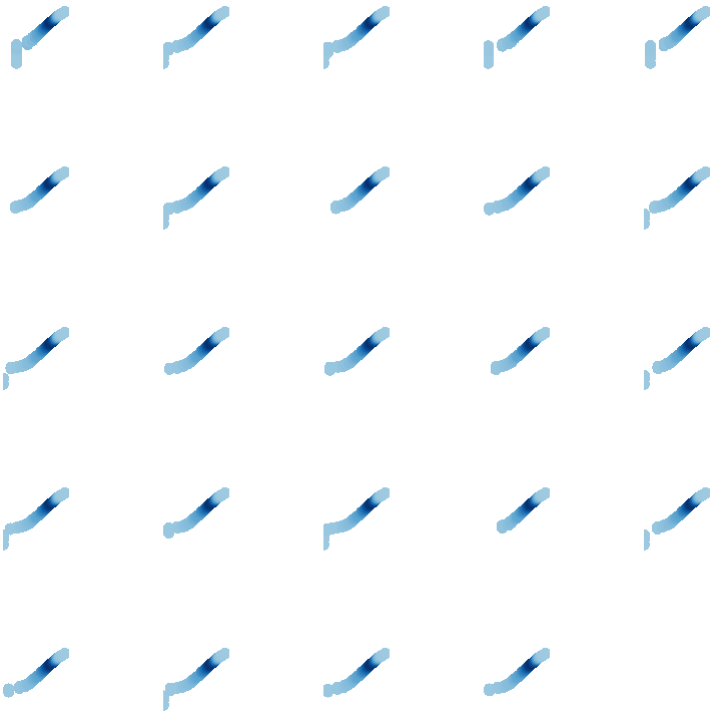




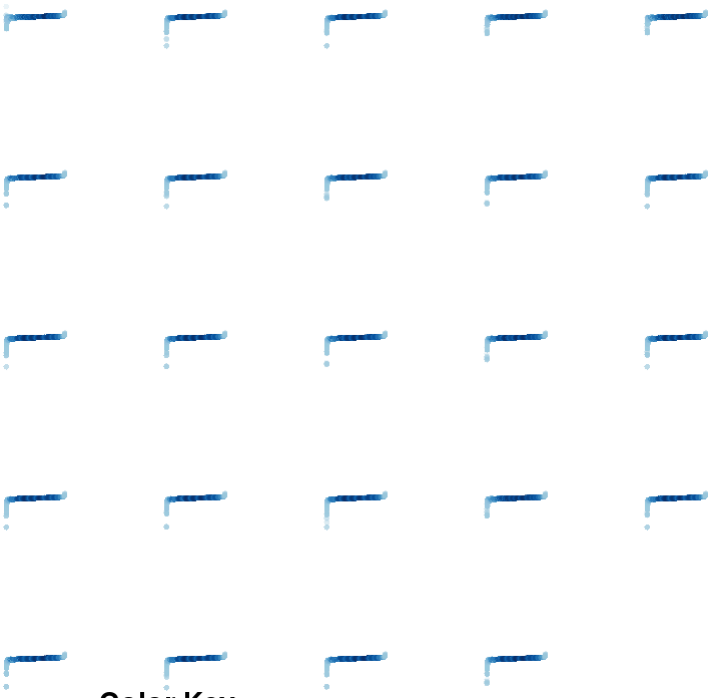
```
## [1] "This plot looks neat if you do position='fill' or position='stack'"

## Using  as id variables
```

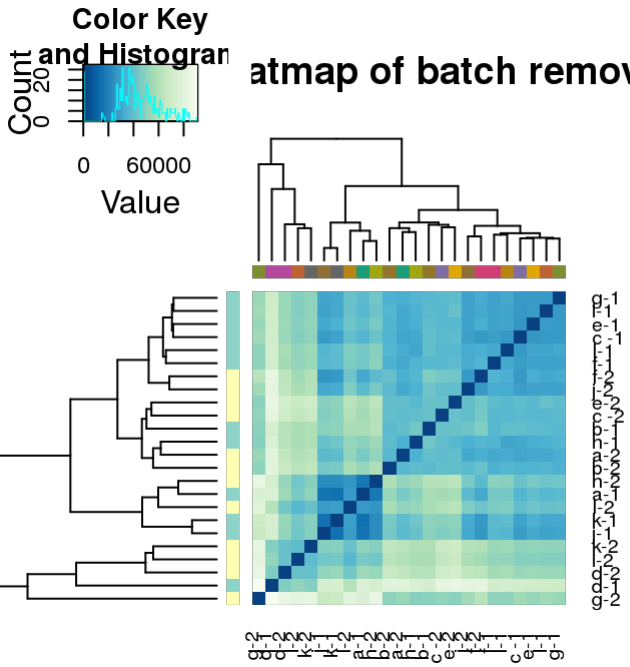


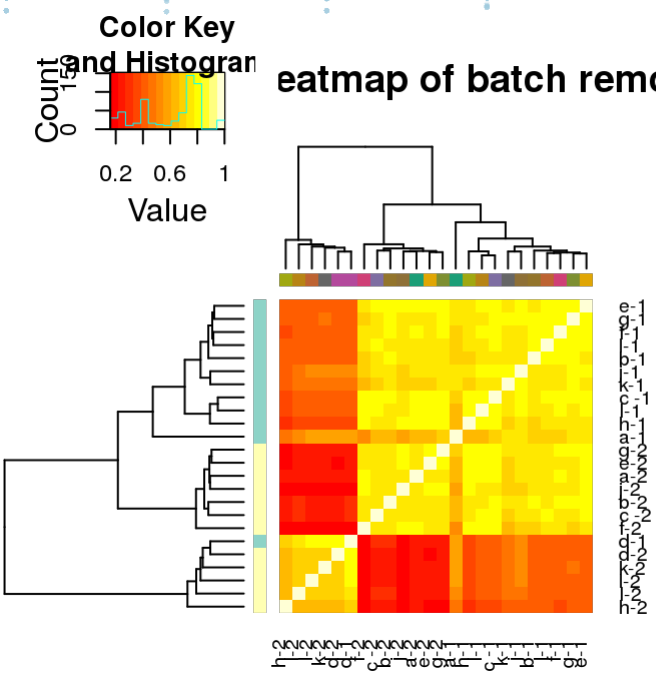
```
## [1] "This plot looks neat if you do position='fill' or position='stack'"
## [1] "Graphing a PCA plot of the normalized data."
##    propVar cumPropVar cond.R2 batch.R2
## 1    35.52      35.52   55.43    14.79
## 2     3.35      38.87   56.83     1.28
## 3     3.22      42.09   37.04     5.14
## 4     3.19      45.28   51.61     4.43
## 5     3.14      48.42   40.21     1.16
## 6     3.08      51.50   31.94     3.16
## 7     3.05      54.55   44.25     2.65
## 8     3.01      57.56   55.16     0.42
## 9     3.01      60.57   43.96     1.14
## 10    2.98      63.55   37.30     5.45
## 11    2.96      66.51   30.58    16.57
## 12    2.95      69.46   64.00     1.17
## 13    2.90      72.36   48.28    10.40
## 14    2.86      75.22   48.58     4.39
## 15    2.85      78.07   56.64    11.18
## 16    2.81      80.88   73.62     0.14
## 17    2.80      83.68   32.59     1.83
## 18    2.79      86.47   36.00     1.02
## 19    2.78      89.25   49.76     0.00
## 20    2.73      91.98   50.16     0.91
## 21    2.70      94.68   62.94     0.32
## 22    2.68      97.36   67.53     2.93
## 23    2.64     100.00   25.61     9.51
## [1] "Printing a qqplot of the normalized data."
```



```
## Using id as id variables
```





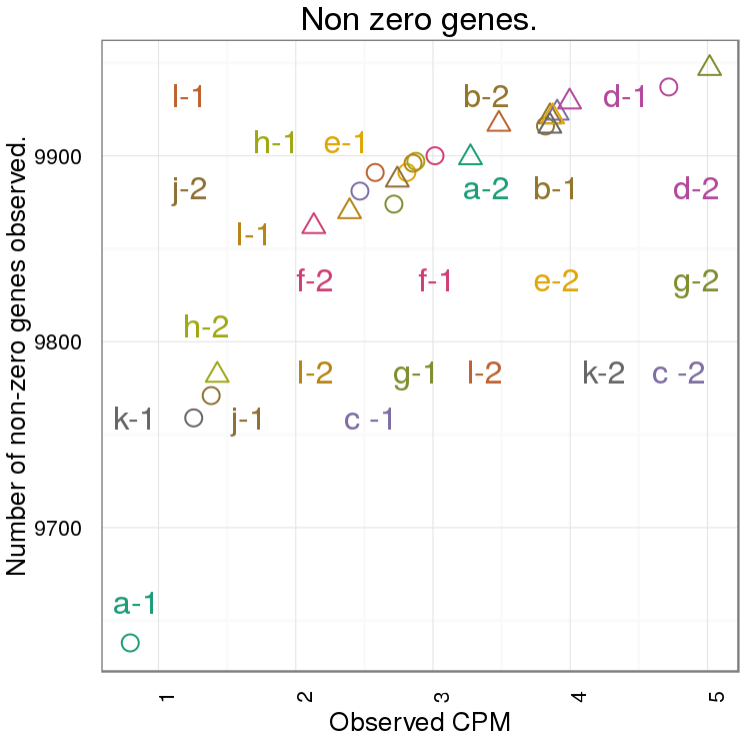



```
##    propVar cumPropVar cond.R2 batch.R2
## 1    40.35      40.35   65.44        0
## 2    21.18      61.53   57.33        0
## 3     4.34      65.87   57.84        0
## 4     3.64      69.51   62.35        0
## 5     3.07      72.58   55.85        0
## 6     3.06      75.64   19.11        0
## 7     2.91      78.55   37.86        0
## 8     2.72      81.27   37.69        0
## 9     2.68      83.95   52.91        0
## 10    2.33      86.28   35.38        0
```

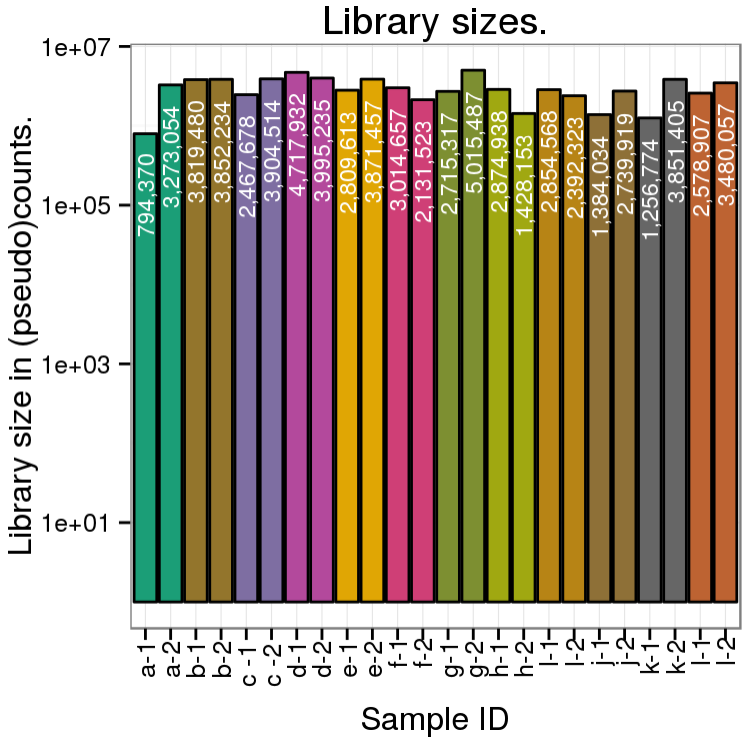
```
## 11    2.00      88.28   49.87        0
## 12    1.65      89.93   53.29        0
## 13    1.56      91.49   68.25        0
## 14    1.51      93.00   33.32        0
## 15    1.34      94.34   44.09        0
## 16    1.26      95.60   48.58        0
## 17    1.19      96.79   59.97        0
## 18    1.07      97.86   45.07        0
## 19    0.97      98.83   55.57        0
## 20    0.58      99.41   57.56        0
## 21    0.36      99.77   54.58        0
## 22    0.23     100.00   48.11        0
```

```
fun
```

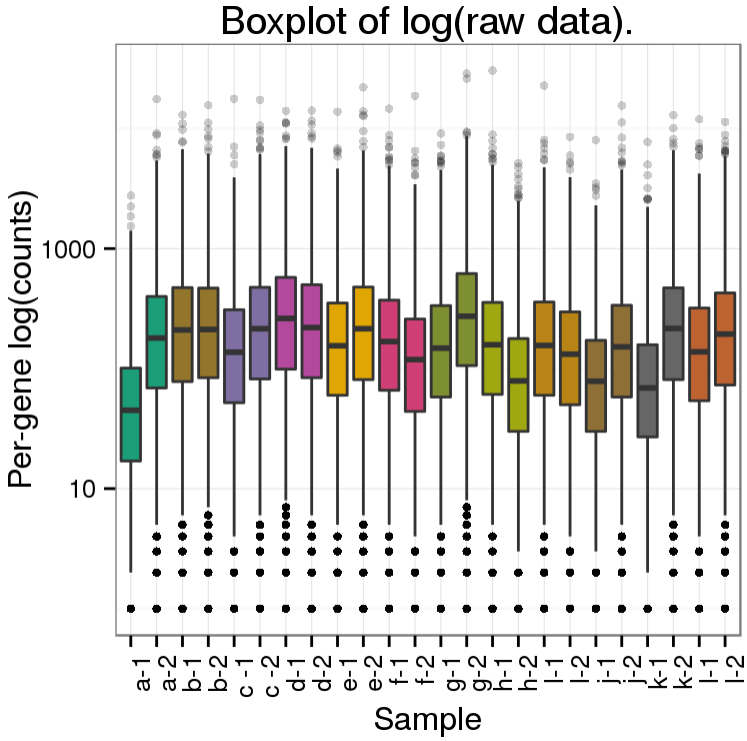
```
## $nonzero
```




```
##
## $libsize
```

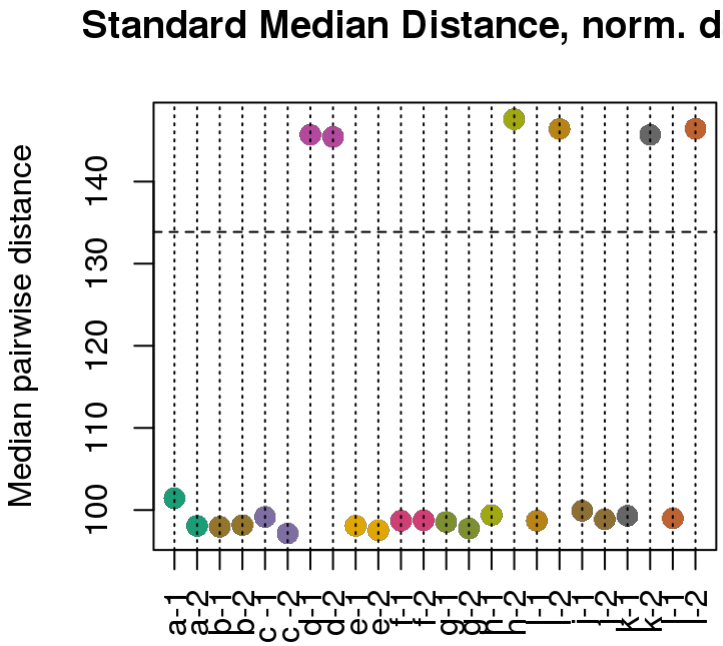



```
##
## $raw_boxplot

## Warning: Removed 1391 rows containing non-finite values (stat_boxplot).
```

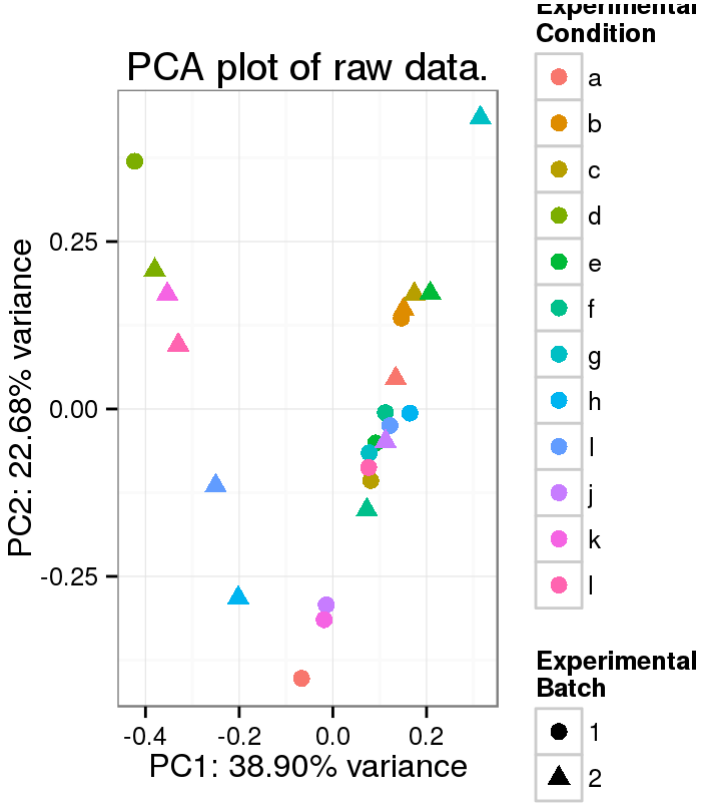



```
##
```

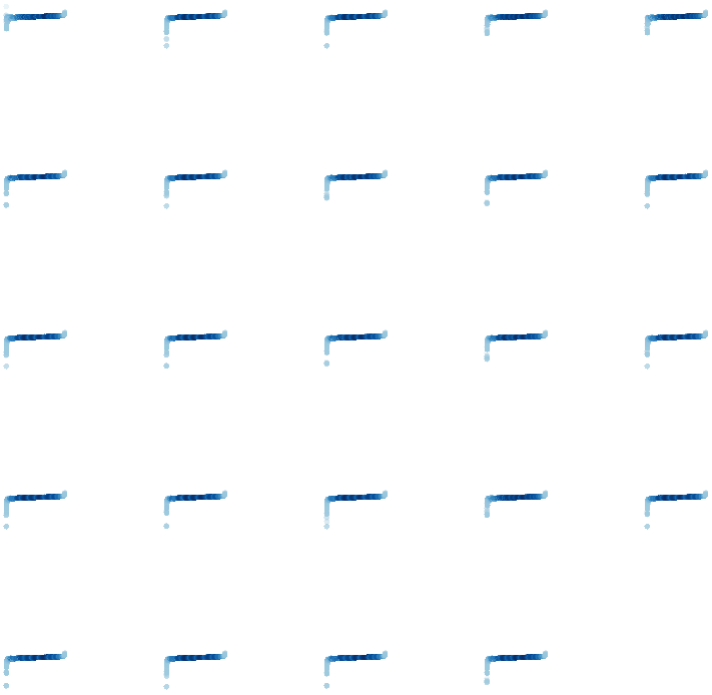
```
## $norm_boxplot
```




```
##
## $raw_corheat
##
## $raw_smc
##
## $norm_corheat
##
## $norm_smc
##
## $raw_disheat
##
## $raw_smd
##
## $norm_disheat
##
## $norm_smd
##
## $raw_pcaplot
```




```
##
## $norm_pcaplot
```



```
##
```

```
## $raw_pcatable
##          SampleID condition batch batch_int         PC1          PC2
## HPGL0406      a-1         a     1         1 -0.06691092 -0.402355331
## HPGL0407      a-2         a     2         2  0.13431332  0.045259223
## HPGL0408      b-1         b     1         1  0.14634248  0.135682093
## HPGL0409      b-2         b     2         2  0.15119716  0.148552727
## HPGL0149     c -1        c      1         1  0.08078548 -0.106755934
## HPGL0150     c -2        c      2         2  0.17403628  0.171498664
## HPGL0147      d-1         d     1         1 -0.42304652  0.369802408
## HPGL0148      d-2         d     2         2 -0.38027775  0.207290683
## HPGL0410      e-1         e     1         1  0.09148588 -0.050776835
## HPGL0411      e-2         e     2         2  0.20806087  0.172566966
## HPGL0412      f-1         f     1         1  0.11211147 -0.005541911
## HPGL0413      f-2         f     2         2  0.07258768 -0.150795269
## HPGL0414      g-1         g     1         1  0.07767734 -0.065569614
## HPGL0416      g-2         g     2         2  0.31491903  0.434733324
## HPGL0415      h-1         h     1         1  0.16438931 -0.006171446
## HPGL0417      h-2         h     2         2 -0.20194956 -0.282064901
## HPGL0418      I-1         I     1         1  0.12188367 -0.024827186
## HPGL0419      I-2         I     2         2 -0.25012222 -0.114756653
## HPGL0420      j-1         j     1         1 -0.01422104 -0.292626128
## HPGL0421      j-2         j     2         2  0.11244544 -0.048430048
## HPGL0422      k-1         k     1         1 -0.01852333 -0.314655028
## HPGL0423      k-2         k     2         2 -0.35343798  0.171561901
## HPGL0424      l-1         l     1         1  0.07632821 -0.087452287
## HPGL0425      l-2         l     2         2 -0.33007430  0.095830582
##
## $norm_pcatable
##          SampleID condition batch batch_int        PC1         PC2
## HPGL0406 HPGL0406         a     1         1 -0.1207486  0.90572396
## HPGL0407 HPGL0407         a     2         2 -0.1204795  0.01720201
## HPGL0408 HPGL0408         b     1         1 -0.1170141 -0.01441490
## HPGL0409 HPGL0409         b     2         2 -0.1141126 -0.07763279
## HPGL0149 HPGL0149        c      1         1 -0.1158684 -0.01919210
## HPGL0150 HPGL0150        c      2         2 -0.1189747 -0.01840768
## HPGL0147 HPGL0147         d     1         1  0.3510610  0.05572912
## HPGL0148 HPGL0148         d     2         2  0.3512098  0.05271144
## HPGL0410 HPGL0410         e     1         1 -0.1212086 -0.12316968
## HPGL0411 HPGL0411         e     2         2 -0.1212779 -0.13158376
## HPGL0412 HPGL0412         f     1         1 -0.1211644  0.05568835
## HPGL0413 HPGL0413         f     2         2 -0.1143935  0.02395079
## HPGL0414 HPGL0414         g     1         1 -0.1174481 -0.04729123
## HPGL0416 HPGL0416         g     2         2 -0.1192606 -0.06767965
## HPGL0415 HPGL0415         h     1         1 -0.1188799  0.03077424
## HPGL0417 HPGL0417         h     2         2  0.3567765  0.04846185
## HPGL0418 HPGL0418         I     1         1 -0.1149888 -0.16489569
## HPGL0419 HPGL0419         I     2         2  0.3537376 -0.13395380
## HPGL0420 HPGL0420         j     1         1 -0.1105709 -0.12138683
## HPGL0421 HPGL0421         j     2         2 -0.1194167  0.02431780
## HPGL0422 HPGL0422         k     1         1 -0.1152913 -0.23787005
## HPGL0423 HPGL0423         k     2         2  0.3534408 -0.03229465
## HPGL0424 HPGL0424         l     1         1 -0.1200275 -0.04302864
## HPGL0425 HPGL0425         l     2         2  0.3549002  0.01824192
##
```

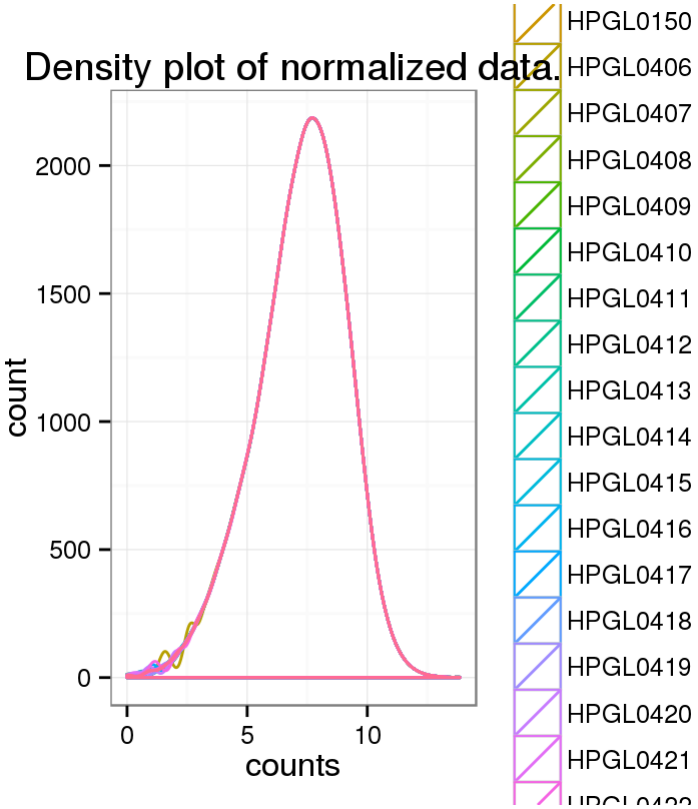
```
## $raw_pcares
##    propVar cumPropVar cond.R2 batch.R2
## 1    38.90      38.90   65.58     2.02
## 2    22.68      61.58   48.03    12.08
## 3     4.18      65.76   55.85     2.90
## 4     3.47      69.23   60.55     1.22
## 5     3.06      72.29   13.77    16.57
## 6     2.91      75.20   51.94     0.01
## 7     2.83      78.03   58.16     2.50
## 8     2.60      80.63   40.48     3.92
## 9     2.57      83.20   29.00     2.97
## 10    2.50      85.70   31.46     6.33
## 11    1.99      87.69   55.96     4.23
## 12    1.64      89.33   50.20     9.96
## 13    1.56      90.89   52.36     0.16
## 14    1.43      92.32   33.43     0.01
## 15    1.27      93.59   45.31     0.17
## 16    1.25      94.84   50.07    16.02
## 17    1.18      96.02   46.71     3.30
## 18    1.12      97.14   60.63     0.07
## 19    1.00      98.14   40.19     0.77
## 20    0.84      98.98   54.10     6.48
## 21    0.45      99.43   53.26     8.16
## 22    0.34      99.77   54.93     0.10
## 23    0.22      99.99   48.03     0.06
##
## $norm_pcares
##    propVar cumPropVar cond.R2 batch.R2
## 1    35.52      35.52   55.43    14.79
## 2     3.35      38.87   56.83     1.28
## 3     3.22      42.09   37.04     5.14
## 4     3.19      45.28   51.61     4.43
## 5     3.14      48.42   40.21     1.16
## 6     3.08      51.50   31.94     3.16
## 7     3.05      54.55   44.25     2.65
## 8     3.01      57.56   55.16     0.42
## 9     3.01      60.57   43.96     1.14
## 10    2.98      63.55   37.30     5.45
## 11    2.96      66.51   30.58    16.57
## 12    2.95      69.46   64.00     1.17
## 13    2.90      72.36   48.28    10.40
## 14    2.86      75.22   48.58     4.39
## 15    2.85      78.07   56.64    11.18
## 16    2.81      80.88   73.62     0.14
## 17    2.80      83.68   32.59     1.83
## 18    2.79      86.47   36.00     1.02
## 19    2.78      89.25   49.76     0.00
## 20    2.73      91.98   50.16     0.91
## 21    2.70      94.68   62.94     0.32
## 22    2.68      97.36   67.53     2.93
## 23    2.64     100.00   25.61     9.51
##
## $raw_pcavar
##  [1] 38.90 22.68  4.18  3.47  3.06  2.91  2.83  2.60  2.57  2.50  1.99
```

```
## [12]  1.64  1.56  1.43  1.27  1.25  1.18  1.12  1.00  0.84  0.45  0.34
## [23]  0.22
##
## $norm_pcavar
##  [1] 35.52  3.35  3.22  3.19  3.14  3.08  3.05  3.01  3.01  2.98  2.96
## [12]  2.95  2.90  2.86  2.85  2.81  2.80  2.79  2.78  2.73  2.70  2.68
## [23]  2.64
##
## $raw_qq
## $raw_qq$logs
##
## $raw_qq$ratios
##
## $raw_qq$medians
## $raw_qq$medians[[1]]
## [1] -1.485
##
## $raw_qq$medians[[2]]
## [1] -0.08172
##
## $raw_qq$medians[[3]]
## [1] 0.0755
##
## $raw_qq$medians[[4]]
## [1] 0.0855
##
## $raw_qq$medians[[5]]
## [1] -0.3545
##
## $raw_qq$medians[[6]]
## [1] 0.09966
##
## $raw_qq$medians[[7]]
## [1] 0.2966
##
## $raw_qq$medians[[8]]
## [1] 0.1217
##
## $raw_qq$medians[[9]]
## [1] -0.2263
##
## $raw_qq$medians[[10]]
## [1] 0.1006
##
## $raw_qq$medians[[11]]
## [1] -0.1516
##
## $raw_qq$medians[[12]]
## [1] -0.4996
##
## $raw_qq$medians[[13]]
## [1] -0.2793
##
## $raw_qq$medians[[14]]
```

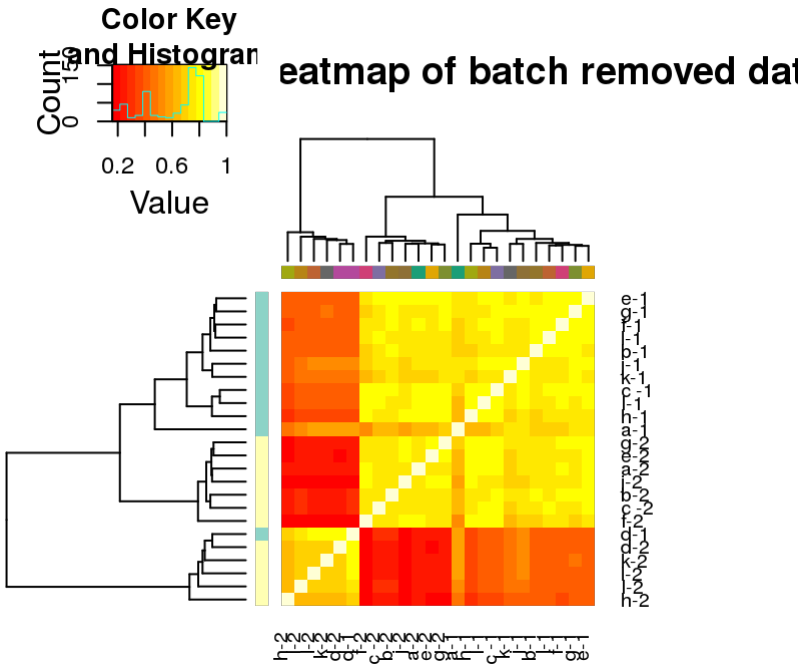
```
## [1] 0.338
##
## $raw_qq$medians[[15]]
## [1] -0.2126
##
## $raw_qq$medians[[16]]
## [1] -0.914
##
## $raw_qq$medians[[17]]
## [1] -0.2255
##
## $raw_qq$medians[[18]]
## [1] -0.3956
##
## $raw_qq$medians[[19]]
## [1] -0.9252
##
## $raw_qq$medians[[20]]
## [1] -0.2499
##
## $raw_qq$medians[[21]]
## [1] -1.044
##
## $raw_qq$medians[[22]]
## [1] 0.1029
##
## $raw_qq$medians[[23]]
## [1] -0.342
##
## $raw_qq$medians[[24]]
## [1] -0.007835
##
##
##
## $norm_qq
## $norm_qq$logs
##
## $norm_qq$ratios
##
## $norm_qq$medians
## $norm_qq$medians[[1]]
## [1] -0.00359
##
## $norm_qq$medians[[2]]
## [1] -0.003924
##
## $norm_qq$medians[[3]]
## [1] -0.004033
##
## $norm_qq$medians[[4]]
## [1] -0.00397
##
## $norm_qq$medians[[5]]
## [1] -0.003868
```

```
##
## $norm_qq$medians[[6]]
## [1] -0.003979
##
## $norm_qq$medians[[7]]
## [1] -0.004028
##
## $norm_qq$medians[[8]]
## [1] -0.004061
##
## $norm_qq$medians[[9]]
## [1] -0.003976
##
## $norm_qq$medians[[10]]
## [1] -0.00401
##
## $norm_qq$medians[[11]]
## [1] -0.003986
##
## $norm_qq$medians[[12]]
## [1] -0.003907
##
## $norm_qq$medians[[13]]
## [1] -0.003956
##
## $norm_qq$medians[[14]]
## [1] -0.004034
##
## $norm_qq$medians[[15]]
## [1] -0.003923
##
## $norm_qq$medians[[16]]
## [1] -0.003823
##
## $norm_qq$medians[[17]]
## [1] -0.003955
##
## $norm_qq$medians[[18]]
## [1] -0.003898
##
## $norm_qq$medians[[19]]
## [1] -0.003805
##
## $norm_qq$medians[[20]]
## [1] -0.003941
##
## $norm_qq$medians[[21]]
## [1] -0.003694
##
## $norm_qq$medians[[22]]
## [1] -0.003973
##
## $norm_qq$medians[[23]]
## [1] -0.003882
```

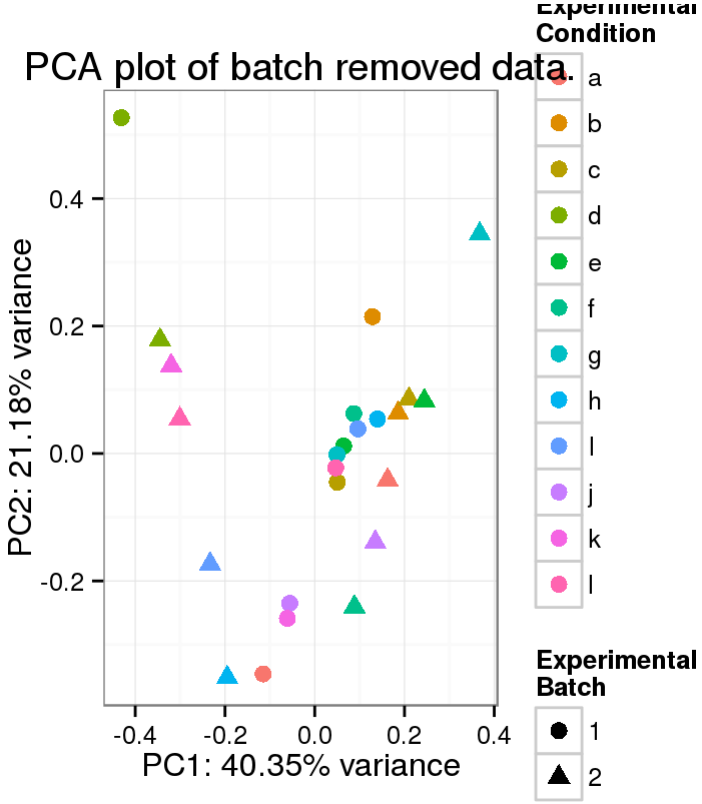
```
##
## $norm_qq$medians[[24]]
## [1] -0.004009
##
##
##
## $raw_density
## [1] "Error in `colnames<-`(`*tmp*`, value = c(\"gene\", \"cond\", \"counts\")) : \n  'names' attribu
## attr(,"class")
## [1] "try-error"
## attr(,"condition")
## <simpleError in `colnames<-`(`*tmp*`, value = c("gene", "cond", "counts")): 'names' attribute [3] mu
##
## $norm_density
```




```
##
## $batch_boxplot

## Warning in scale$trans$trans(x): NaNs produced

## Warning: Removed 3428 rows containing non-finite values (stat_boxplot).
```




```
##
## $batch_disheat
##
## $batch_corheat
##
## $batch_pcaplot
```



```
##
## $batch_pcatable
##          SampleID condition batch batch_int         PC1          PC2
## HPGL0406 HPGL0406         a     1         1 -0.11491095 -0.345933794
## HPGL0407 HPGL0407         a     2         2  0.16235698 -0.041669613
## HPGL0408 HPGL0408         b     1         1  0.12865889  0.214808793
## HPGL0409 HPGL0409         b     2         2  0.18578957  0.063069284
## HPGL0149 HPGL0149        c      1         1  0.05025117 -0.045143040
## HPGL0150 HPGL0150        c      2         2  0.21001438  0.085441344
## HPGL0147 HPGL0147         d     1         1 -0.43099566  0.526947894
## HPGL0148 HPGL0148         d     2         2 -0.34480826  0.178184416
## HPGL0410 HPGL0410         e     1         1  0.06445727  0.011750093
## HPGL0411 HPGL0411         e     2         2  0.24431995  0.082753685
## HPGL0412 HPGL0412         f     1         1  0.08698558  0.062738203
## HPGL0413 HPGL0413         f     2         2  0.08851754 -0.240609408
## HPGL0414 HPGL0414         g     1         1  0.04958653 -0.001669822
## HPGL0416 HPGL0416         g     2         2  0.36767050  0.344440442
## HPGL0415 HPGL0415         h     1         1  0.13988732  0.054193952
## HPGL0417 HPGL0417         h     2         2 -0.19505916 -0.351635919
## HPGL0418 HPGL0418         I     1         1  0.09610493  0.038693897
## HPGL0419 HPGL0419         I     2         2 -0.23310810 -0.173407590
## HPGL0420 HPGL0420         j     1         1 -0.05558380 -0.235089831
## HPGL0421 HPGL0421         j     2         2  0.13497146 -0.139018139
## HPGL0422 HPGL0422         k     1         1 -0.06104296 -0.258840219
## HPGL0423 HPGL0423         k     2         2 -0.31995049  0.137980932
## HPGL0424 HPGL0424         l     1         1  0.04660168 -0.022456127
## HPGL0425 HPGL0425         l     2         2 -0.30071438  0.054470565
##
## $batch_pcares
##    propVar cumPropVar cond.R2 batch.R2
## 1    40.35      40.35   65.44        0
## 2    21.18      61.53   57.33        0
## 3     4.34      65.87   57.84        0
## 4     3.64      69.51   62.35        0
## 5     3.07      72.58   55.85        0
## 6     3.06      75.64   19.11        0
## 7     2.91      78.55   37.86        0
## 8     2.72      81.27   37.69        0
## 9     2.68      83.95   52.91        0
## 10    2.33      86.28   35.38        0
## 11    2.00      88.28   49.87        0
## 12    1.65      89.93   53.29        0
## 13    1.56      91.49   68.25        0
## 14    1.51      93.00   33.32        0
## 15    1.34      94.34   44.09        0
## 16    1.26      95.60   48.58        0
## 17    1.19      96.79   59.97        0
## 18    1.07      97.86   45.07        0
## 19    0.97      98.83   55.57        0
## 20    0.58      99.41   57.56        0
## 21    0.36      99.77   54.58        0
## 22    0.23     100.00   48.11        0
##
## $batch_pcavar
```

```
##  [1] 40.35 21.18  4.34  3.64  3.07  3.06  2.91  2.72  2.68  2.33  2.00
## [12]  1.65  1.56  1.51  1.34  1.26  1.19  1.07  0.97  0.58  0.36  0.23
```

```
## The following are some examples of other ways to make use of these plots:

##fun_boxplot = my_boxplot(df=fun)
##print(fun_boxplot)
##log_boxplot = my_boxplot(df=fun, scale="log")
##print(log_boxplot)
##my_corheat(df=fun, colors=my_colors)
##my_disheat(df=fun, colors=my_colors)
##my_smc(df=fun, colors=my_colors)
##my_libsize(df=fun)
##my_qq_all(df=fun)
```

Normalizing data

RNAseq data must be normalized. Here is one easy method:

```
## normalize_expt will do this on the expt class, replace the expressionset therein, and
## make a backup of the data inside the expt class.
norm_expt = normalize_expt(all_expt)
```

```
## [1] "This function defaults to using the original expressionset for normalization."
```

```
head(exprs(norm_expt$expressionset))
```

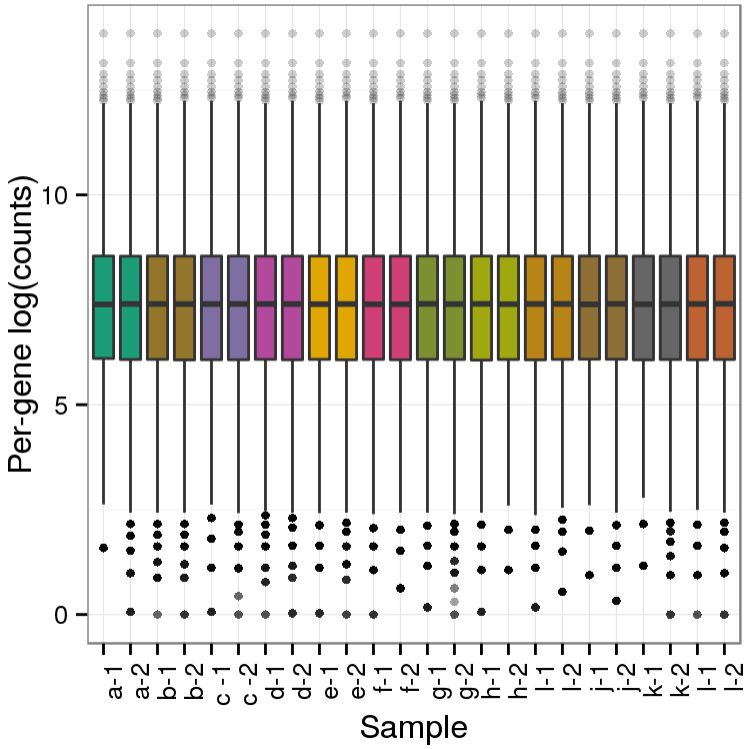
```
##           HPGL0406  HPGL0407  HPGL0408 HPGL0409 HPGL0149 HPGL0150 HPGL0147
## gene_1_F  5.277675  7.136137  7.984537 8.745534 8.222795 6.158610 7.002112
## gene_2_T  8.882579 10.240344  9.477674 8.290211 6.854609 8.886459 7.682117
## gene_3_F  4.333901  5.402302  3.894818 5.613483 5.571121 4.909893 4.996238
## gene_4_F  4.579944  5.842350  5.815383 4.698126 5.171594 3.662965 4.634206
## gene_5_F  5.630570  7.080373  6.719389 6.703904 7.530081 6.121534 7.421890
## gene_6_F 10.772143  9.766805 10.522132 8.964822 9.490308 9.277675 9.431367
##           HPGL0148 HPGL0410 HPGL0411  HPGL0412  HPGL0413  HPGL0414
## gene_1_F  7.349834 6.422766 7.955892  7.903882  6.677132  7.662075
## gene_2_T  7.823633 8.765010 9.096825  9.443980  9.001877 10.013904
## gene_3_F  5.321928 5.637832 4.809500  6.203756  5.370687  5.241586
## gene_4_F  4.853829 5.014950 4.357552  4.579944  3.954196  4.641450
## gene_5_F  6.048487 7.634206 7.442598  7.005157  8.040290  6.893302
## gene_6_F 10.088070 9.692107 9.934213 10.129337 10.482724  9.604476
##          HPGL0416 HPGL0415 HPGL0417 HPGL0418 HPGL0419  HPGL0420 HPGL0421
## gene_1_F 8.421364 8.599215 7.591211 7.252271 7.667407  8.006560 6.399456
## gene_2_T 9.660033 8.061371 7.989986 9.630950 7.773139  9.268152 7.742590
## gene_3_F 5.685333 5.562242 5.353147 5.367779 5.127564  5.606097 3.122397
## gene_4_F 4.377934 4.952256 5.191471 4.849666 4.412217  5.524215 5.931230
## gene_5_F 6.352411 7.454642 6.717676 7.140830 7.208234  7.609025 6.399456
## gene_6_F 8.941048 8.323336 9.055508 9.260233 9.232621 10.137925 9.850708
##          HPGL0422 HPGL0423 HPGL0424 HPGL0425
## gene_1_F 7.097593 7.684895 8.136350 7.349466
## gene_2_T 8.747564 7.378656 8.468963 7.948489
## gene_3_F 6.815917 3.646259 5.474382 5.366322
```

```
## gene_4_F 4.033423 6.162391 4.288482 5.929752
## gene_5_F 7.870878 6.767633 6.207014 6.863670
## gene_6_F 9.463780 9.681897 8.124660 9.840286
```

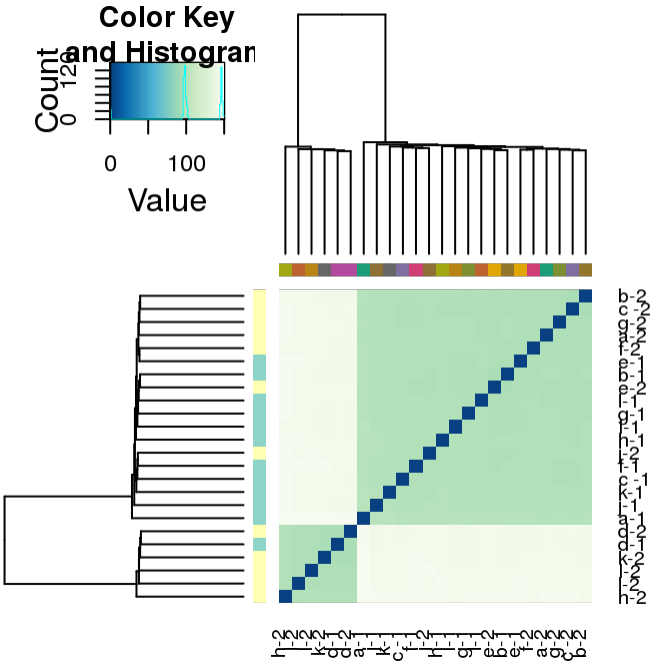
```
## size factor, tmm, rle, upperQuartile all require a design matrix.
norm_boxplot = my_boxplot(expt=norm_expt)
```

```
## Using id as id variables
```

```
print(norm_boxplot)
```




```
norm_disheat = my_disheat(expt=norm_expt)
```

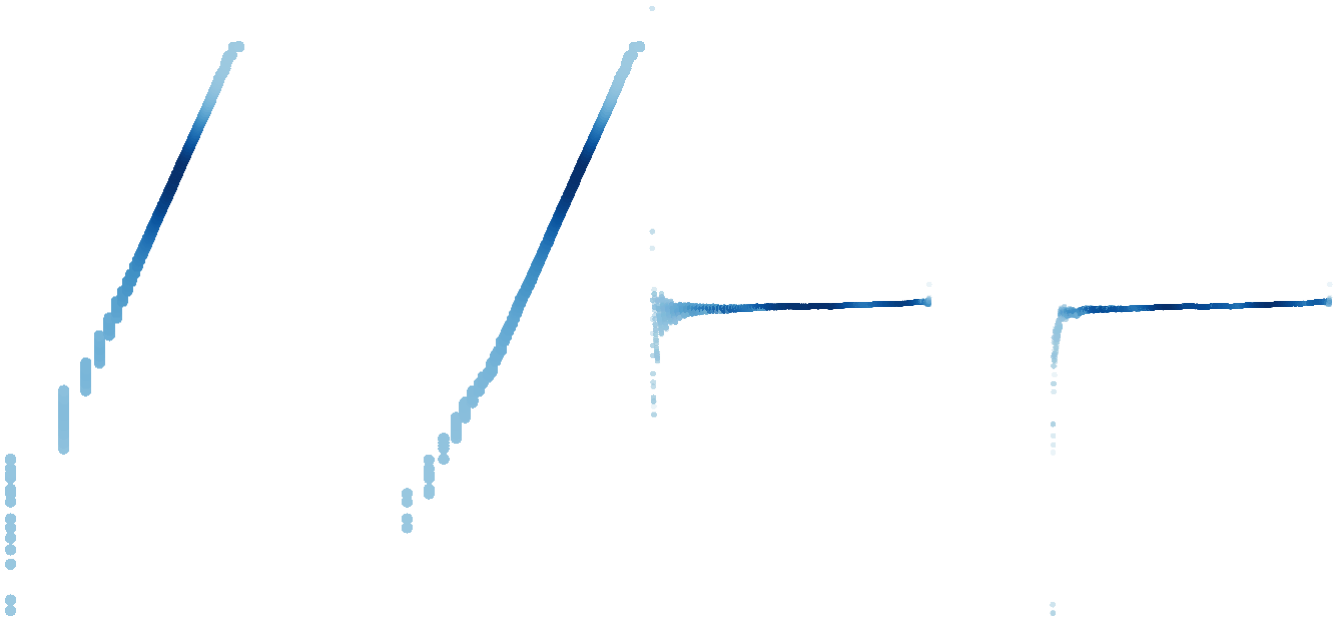



```
print(norm_disheat)
```

Voom/limma etc

There are a couple ways to call limma using the expt class. In some cases, it might be useful to pull out a subset of the data and only compare the samples of specific conditions/batches/etc.

```
## el_subset means to pull out only those samples which represent 'Early Log' growth.
el_subset = expt_subset(norm_expt, "stage=='EL'")
## Conversely, one may pull samples which are early log and also wild type
elwt_subset = expt_subset(norm_expt, "stage=='EL'&type=='WT'")
## These subsets may be characterized with the plots as above
## Here is a qq plot as an example.
elwt_qqs = my_qq_all(expt=elwt_subset)
```



```
## Simple comparison will take the first condition as control and the second
## as experimental, if we look at el_subset, we will see that means conditions
## 'a' and 'b'.  Thus performing simple_comparison will look for differentially
## expressed genes between them.
head(el_subset$design)
```

```
##            sample stage type condition batch   color
## HPGL0406 HPGL0406    EL   WT         a     1 #1B9E77
## HPGL0407 HPGL0407    EL   WT         a     2 #1B9E77
## HPGL0408 HPGL0408    EL  mga         b     1 #93752C
## HPGL0409 HPGL0409    EL  mga         b     2 #93752C
##                                                    counts
## HPGL0406  processed_data/count_tables/wt/el/HPGL0406.count.gz
## HPGL0407  processed_data/count_tables/wt/el/HPGL0407.count.gz
## HPGL0408 processed_data/count_tables/mga/el/HPGL0408.count.gz
## HPGL0409 processed_data/count_tables/mga/el/HPGL0409.count.gz
##                                                intercounts
## HPGL0406  data/count_tables/wt/el/HPGL0406_inter.count.gz
## HPGL0407  data/count_tables/wt/el/HPGL0407_inter.count.gz
## HPGL0408 data/count_tables/mga/el/HPGL0408_inter.count.gz
## HPGL0409 data/count_tables/mga/el/HPGL0409_inter.count.gz
```

```
ab_comparison = simple_comparison(el_subset)
```

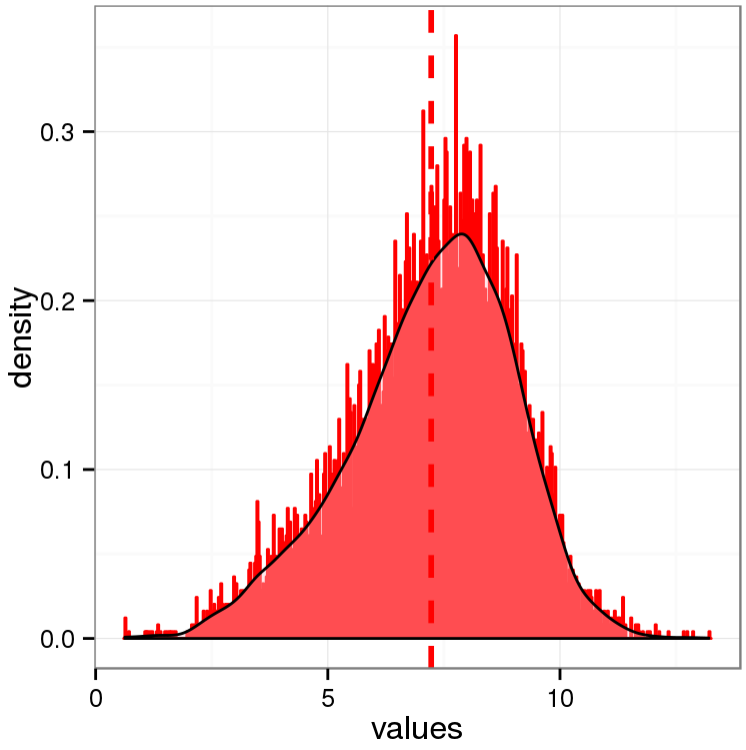
```
## [1] "No binwidth nor bins provided, setting it to 0.0268657804405248 in order to have 500 bins."
```

```
## A summary of the data will show the data provided:
## The following plots and pieces of data show the output provided by simple_comparison()
## This function isn't really intended to be used, but provides a reference point for performing other a
summary(ab_comparison)
```

```
##                       Length Class         Mode
```

```
## amean_histogram           9  gg            list
## coef_amean_cor            9  htest         list
## coefficient_scatter       9  gg            list
## coefficient_x             9  gg            list
## coefficient_y             9  gg            list
## coefficient_both          4  -none-        list
## coefficient_lm           22  lmrob         list
## coefficient_lmsummary    15  summary.lmrob list
## coefficient_weights    9802  -none-        numeric
## comparisons            9802  MArrayLM      list
## contrasts              9802  MArrayLM      list
## contrast_histogram        9  gg            list
## downsignificant           6  data.frame    list
## fit                   29406  MArrayLM      list
## ma_plot                   9  gg            list
## psignificant              6  data.frame    list
## pvalue_histogram          9  gg            list
## table                     6  data.frame    list
## upsignificant             6  data.frame    list
## volcano_plot              9  gg            list
## voom_data             39208  EList         list
## voom_plot                 9  gg            list
```

```
print(ab_comparison$amean_histogram)  ## A histogram of the per-gene mean values
```

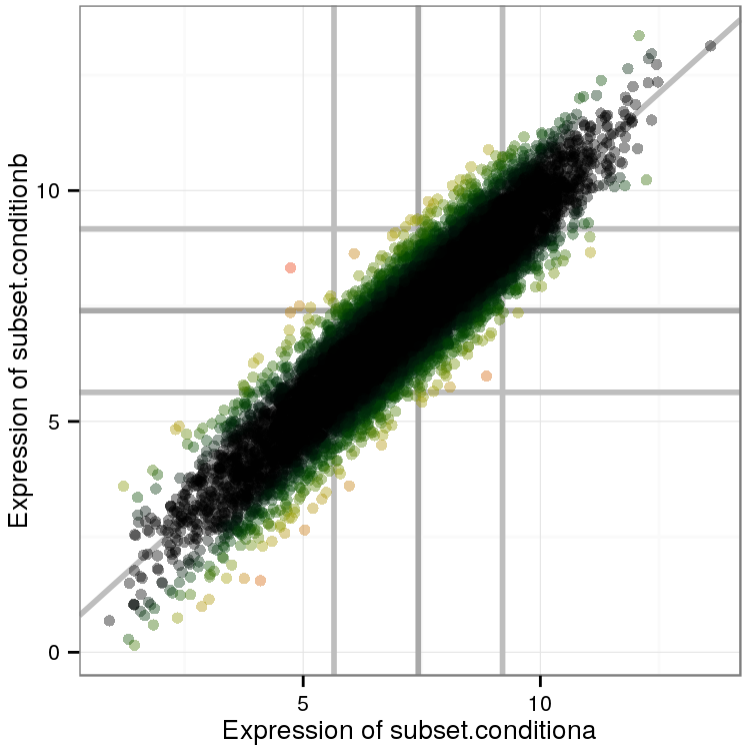



```
print(ab_comparison$coef_amean_cor)   ## The correlation of the means (should not be significant)
```

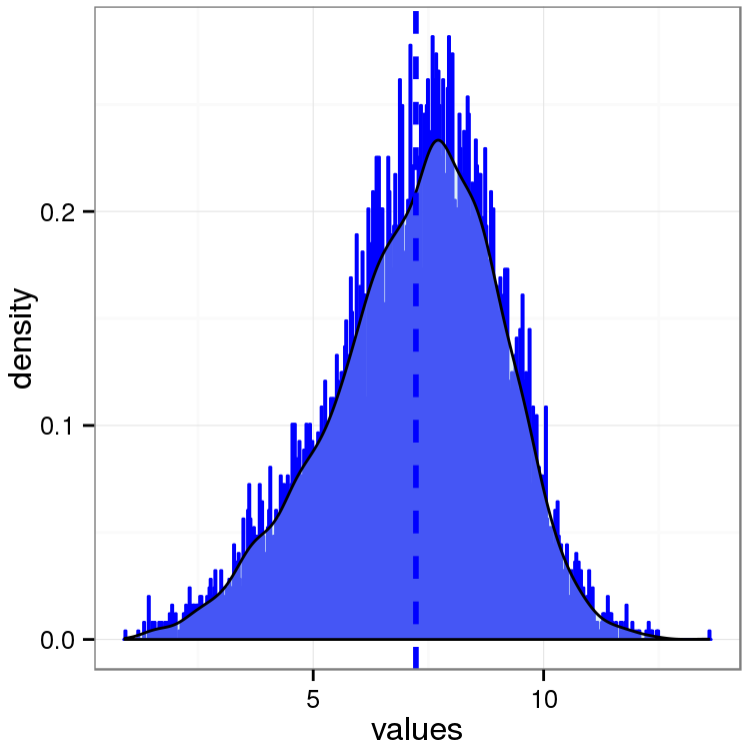
```
##
##  Pearson's product-moment correlation
```

```
##
## data:  cond_contrasts$coefficients and cond_contrasts$Amean
## t = -1.1163, df = 9800, p-value = 0.2643
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  -0.031065393  0.008523674
## sample estimates:
##         cor
## -0.01127528
```

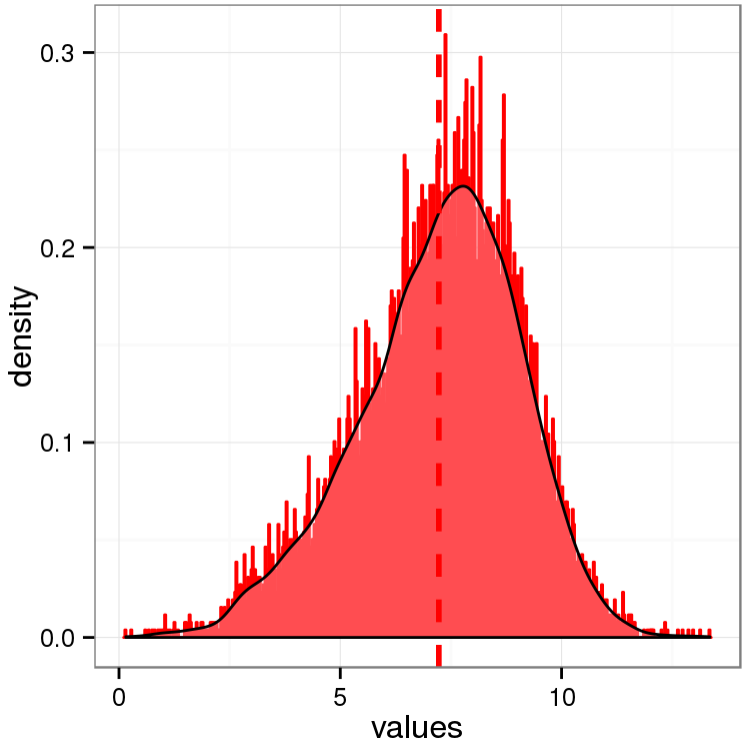
```
print(ab_comparison$coefficient_scatter) ## A scatter plot of condition b with respect to a
```




```
print(ab_comparison$coefficient_x) ## A histogram of the gene abundances of a
```

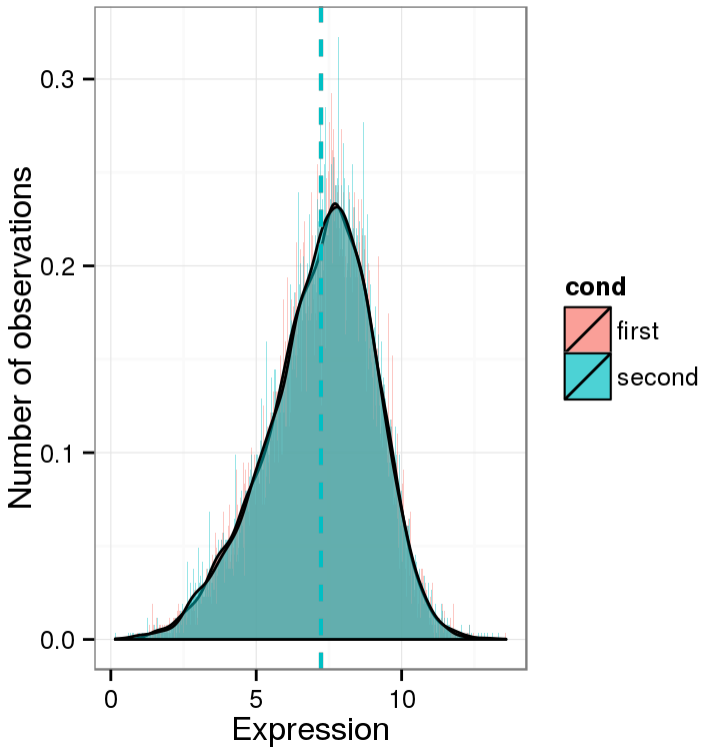



```
print(ab_comparison$coefficient_y) ## A histogram of the gene abundances of b
```




```
print(ab_comparison$coefficient_both) ## A histogram of the gene abundances of a and b
```

```
## $plot
```




```
##
## $data_summary
##      first              second
##  Min.   : 0.9137   Min.   : 0.1542
##  1st Qu.: 6.0854   1st Qu.: 6.1165
##  Median : 7.4264   Median : 7.3985
##  Mean   : 7.2285   Mean   : 7.2269
##  3rd Qu.: 8.5245   3rd Qu.: 8.5116
##  Max.   :13.5871   Max.   :13.3510
##
## $uncor_t
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  play_all$expression and play_all$cond
##
##        first
## second 0.95
##
## P value adjustment method: none
##
## $bon_t
##
##  Pairwise comparisons using t tests with pooled SD
##
## data:  play_all$expression and play_all$cond
##
##        first
## second 0.95
##
```

```
## P value adjustment method: bonferroni
```

```
## Note to self, I keep meaning to change the colors of that to match the others
print(ab_comparison$coefficient_lm) ## The description of the line which describes the relationship
```

```
##
## Call:
## robustbase::lmrob(formula = second ~ first, data = df, method = "SMDM")
##
## Coefficients:
## (Intercept)        first
##      0.5345       0.9262
```

```
## of all of the genes in a to those in b
print(ab_comparison$coefficient_lmsummary) ## A summary of the robust linear model in coefficient_lm
```

```
##
## Call:
## robustbase::lmrob(formula = second ~ first, data = df, method = "SMDM")
##  \--> method = "SMDM"
## Residuals:
##       Min        1Q    Median        3Q       Max
## -2.780264 -0.457062  0.002414  0.462027  3.406376
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept) 0.534502   0.029254   18.27   <2e-16 ***
## first       0.926189   0.003917  236.48   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Robust residual standard error: 0.6852
## Multiple R-squared:  0.857,  Adjusted R-squared:  0.857
## Convergence in 6 IRWLS iterations
##
## Robustness weights:
##  6829 weights are ~= 1. The remaining 2973 ones are summarized as
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
## 0.07759 0.81920 0.92680 0.87060 0.97850 0.99900
## Algorithmic parameters:
##       tuning.chi1       tuning.chi2       tuning.chi3       tuning.chi4
##        -5.000e-01         1.500e+00                NA         5.000e-01
##                bb       tuning.psi1       tuning.psi2       tuning.psi3
##         5.000e-01        -5.000e-01         1.500e+00         9.500e-01
##       tuning.psi4        refine.tol           rel.tol         solve.tol
##                NA         1.000e-07         1.000e-07         1.000e-07
##       eps.outlier             eps.x warn.limit.reject warn.limit.meanrw
##         1.020e-05         2.471e-11         5.000e-01         5.000e-01
##      nResample         max.it       best.r.s       k.fast.s          k.max
##            500             50              2              1            200
##    maxit.scale      trace.lev            mts     compute.rd      numpoints
##            200              0           1000              0             10
## fast.s.large.n
```

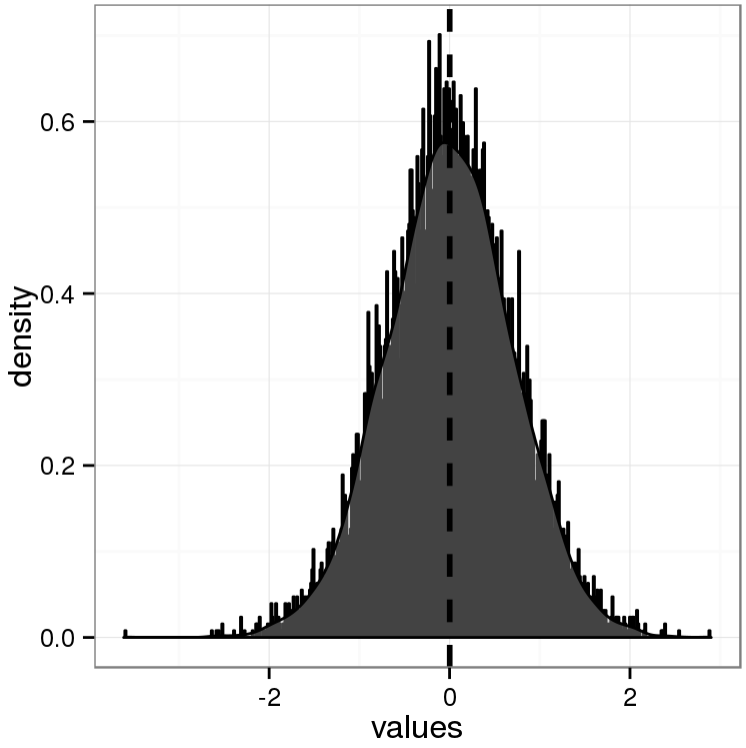
```
##           2000
##                    psi           subsampling                    cov
##                 "lqq"         "nonsingular"              ".vcov.w"
## compute.outlier.stats
##                "SMDM"
## seed : int(0)
```

```
## This has some neat things like the R-squared value and the parameters used to arrive at the linear m
## ab_comparison$coefficient_weights ## a list of weights by gene, bigger weights mean closer to the li
## ab_comparison$comparisons ## the raw output from limma
print(ab_comparison$contrasts)  ## The output from limma's makeContrasts()
```

```
## An object of class "MArrayLM"
## $coefficients
##    gene_1_F    gene_2_T    gene_3_F    gene_4_F    gene_5_F
## -2.13621339  0.68041368  0.08316415 -0.03970033 -0.34221076
## 9797 more rows ...
##
## $stdev.unscaled
##  gene_1_F  gene_2_T  gene_3_F  gene_4_F  gene_5_F
## 0.4629618 0.4359593 0.5227622 0.4921457 0.4651537
## 9797 more rows ...
##
## $sigma
## [1] 1.1999525 2.9211909 0.6403744 2.4216261 1.5848929
## 9797 more elements ...
##
## $df.residual
## [1] 1 1 1 1 1
## 9797 more elements ...
##
## $cov.coefficients
##                    Contrasts
## Contrasts           changed_v_control
##   changed_v_control                 1
##
## $Amean
## gene_1_F gene_2_T gene_3_F gene_4_F gene_5_F
## 7.285971 9.222702 4.811126 5.233951 6.533559
## 9797 more elements ...
##
## $method
## [1] "ls"
##
## $design
##   changed control subset_batch2
## 1       1       0             0
## 2       1       0             1
## 3       0       1             0
## 4       0       1             1
## attr(,"assign")
## [1] 1 1 2
## attr(,"contrasts")
## attr(,"contrasts")$`subset$condition`
```

```
## [1] "contr.treatment"
##
## attr(,"contrasts")$`subset$batch`
## [1] "contr.treatment"
##
##
## $contrasts
##                Contrasts
## Levels          changed_v_control
##   changed                       1
##   control                      -1
##   subset_batch2                 0
```

```
print(ab_comparison$contrast_histogram)  ## A histogram of the values of b-a for each gene
```




```
head(ab_comparison$downsignificant)  ## The list of genes which are significantly down in b vs a
```

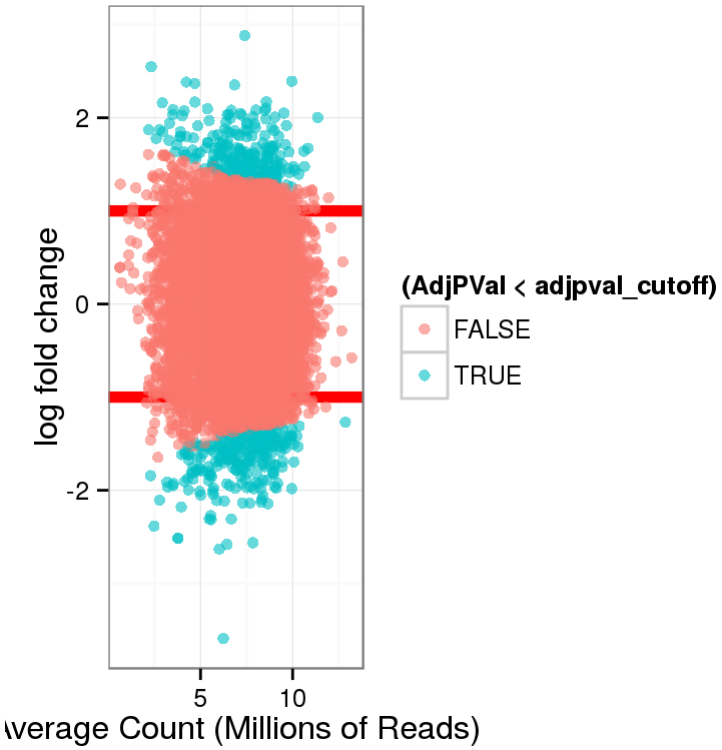
```
##                 logFC  AveExpr         t      P.Value   adj.P.Val
## gene_9453_F -3.591394 6.233951 -4.990457 6.127194e-07 0.006005876
## gene_5901_F -2.630602 6.018968 -3.642978 2.708929e-04 0.490763527
## gene_2096_F -2.580912 6.438045 -3.616247 3.004062e-04 0.490763527
## gene_175_F  -2.563630 7.845211 -3.754706 1.745489e-04 0.490763527
## gene_2815_F -2.515872 3.777812 -3.099054 1.946890e-03 0.700272109
## gene_2131_T -2.513584 3.769331 -3.088450 2.017658e-03 0.700272109
##                       B
## gene_9453_F  1.9895322
## gene_5901_F -1.2882390
## gene_2096_F -1.3115324
## gene_175_F  -0.8958588
```

```
## gene_2815_F -2.5330779
## gene_2131_T -2.5542873
```

```
dim(ab_comparison$downsignificant)
```

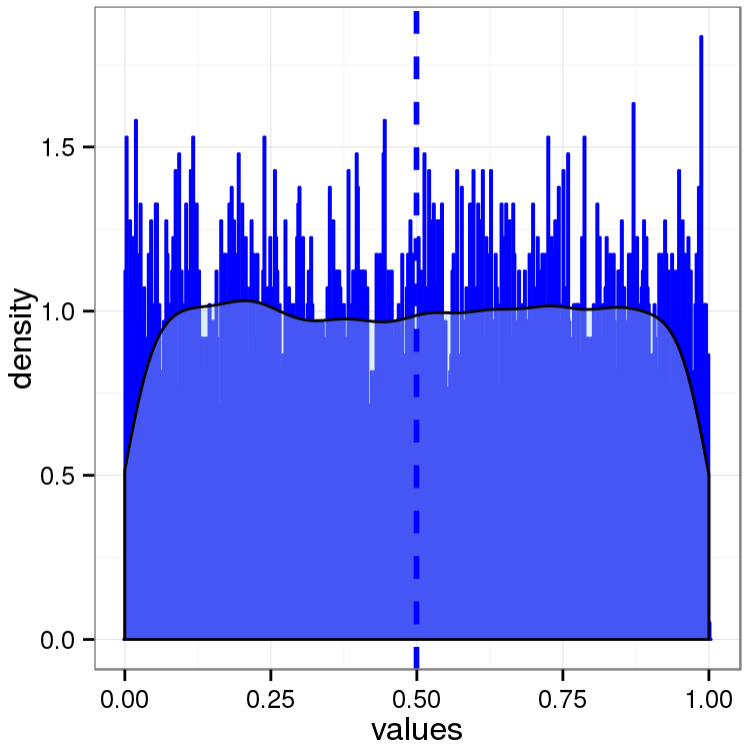
```
## [1] 1910    6
```

```
## ab_comparison$fit ## the result from lmFit()
print(ab_comparison$ma_plot)  ## An ma plot of b vs a
```



verage Count (Millions of Reads)

```
print(ab_comparison$pvalue_histogram) ## A histogram of the p-values, one would hope to see a spike in
```




```
head(ab_comparison$table) ## The full contrast table
```

```
##                 logFC  AveExpr         t      P.Value   adj.P.Val
## gene_9453_F -3.591394 6.233951 -4.990457 6.127194e-07 0.006005876
## gene_5460_F  2.883254 7.402550  4.220374 2.460958e-05 0.120611539
## gene_5901_F -2.630602 6.018968 -3.642978 2.708929e-04 0.490763527
## gene_2096_F -2.580912 6.438045 -3.616247 3.004062e-04 0.490763527
## gene_175_F  -2.563630 7.845211 -3.754706 1.745489e-04 0.490763527
## gene_7746_F  2.548270 2.323350  2.963975 3.044235e-03 0.700272109
##                       B
## gene_9453_F  1.9895322
## gene_5460_F  0.1931333
## gene_5901_F -1.2882390
## gene_2096_F -1.3115324
## gene_175_F  -0.8958588
## gene_7746_F -2.8365400
```

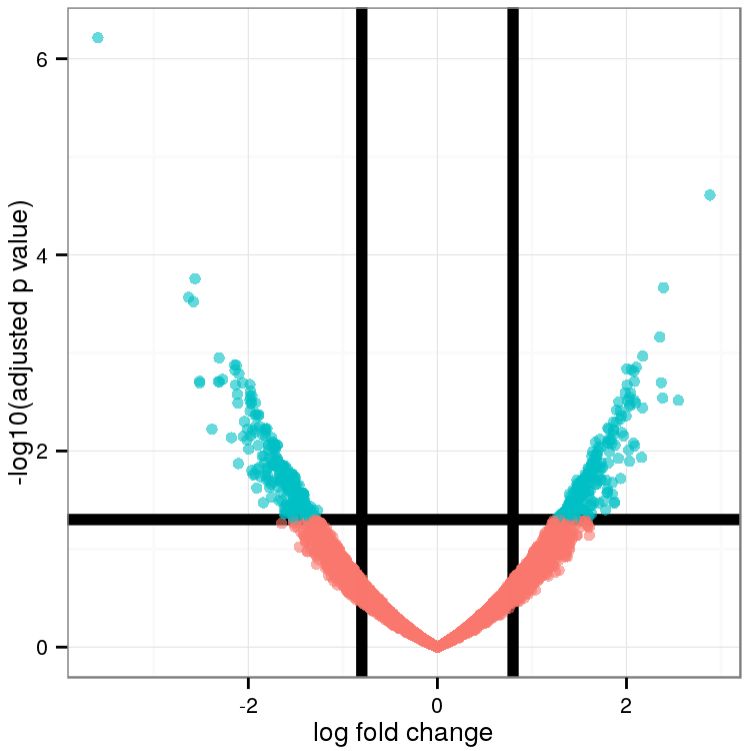
```
head(ab_comparison$upsignificant)  ## The list of genes which are significantly up in b vs a
```

```
##                logFC  AveExpr        t      P.Value adj.P.Val          B
## gene_5460_F 2.883254 7.402550 4.220374 2.460958e-05 0.1206115  0.1931333
## gene_7746_F 2.548270 2.323350 2.963975 3.044235e-03 0.7002721 -2.8365400
## gene_5451_T 2.391506 9.955092 3.701126 2.158206e-04 0.4907635 -0.8653010
## gene_2727_F 2.383002 4.224763 2.980152 2.888122e-03 0.7002721 -2.6885611
## gene_2120_T 2.368166 4.681907 3.089503 2.010527e-03 0.7002721 -2.4407321
## gene_6055_F 2.352462 6.844305 3.395269 6.883327e-04 0.7002721 -1.6834625
```

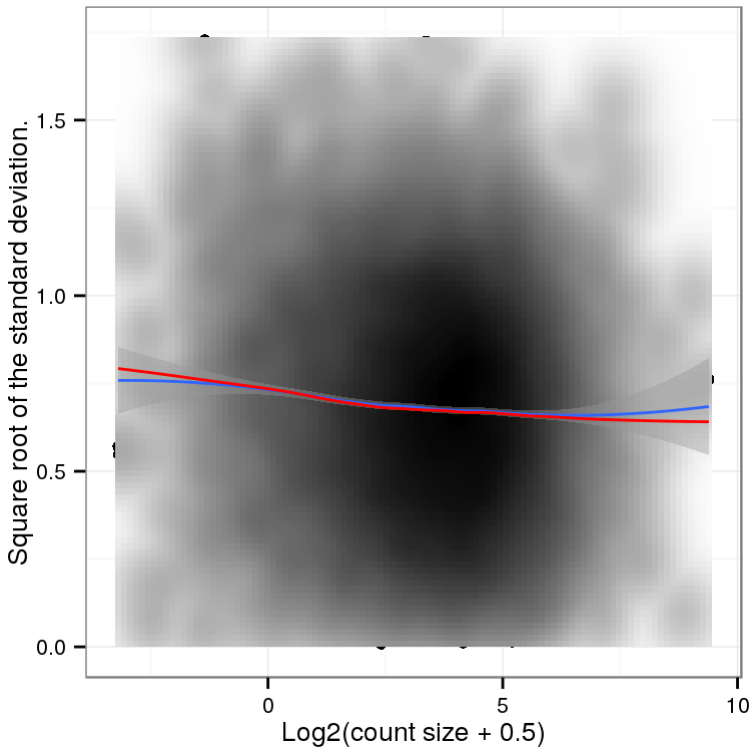
```
dim(ab_comparison$upsignificant)
```

```
## [1] 1902    6
```

```
print(ab_comparison$volcano_plot) ## A Volcano plot of b vs a
```




```
## ab_comparison$voom_data  ## The output from voom()
print(ab_comparison$voom_plot) ## A ggplot2 version of the mean/variance trend provided by voom()
```



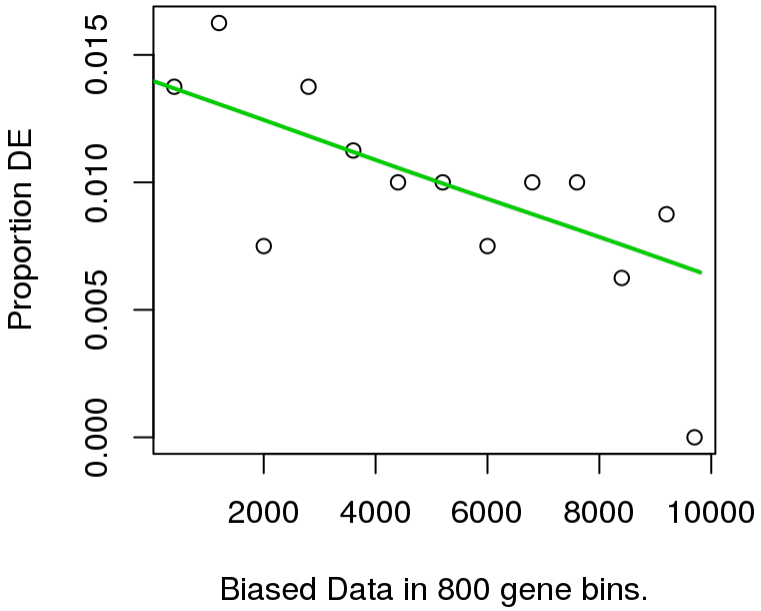

```
## The data structure ab_comparison$comparisons contains the output from eBayes() which comprises the la
## limma step...
funkytown = write_limma(data=ab_comparison$comparisons, excel=FALSE, csv=FALSE)
```

```
## [1] "Printing table: changed_v_control"
```

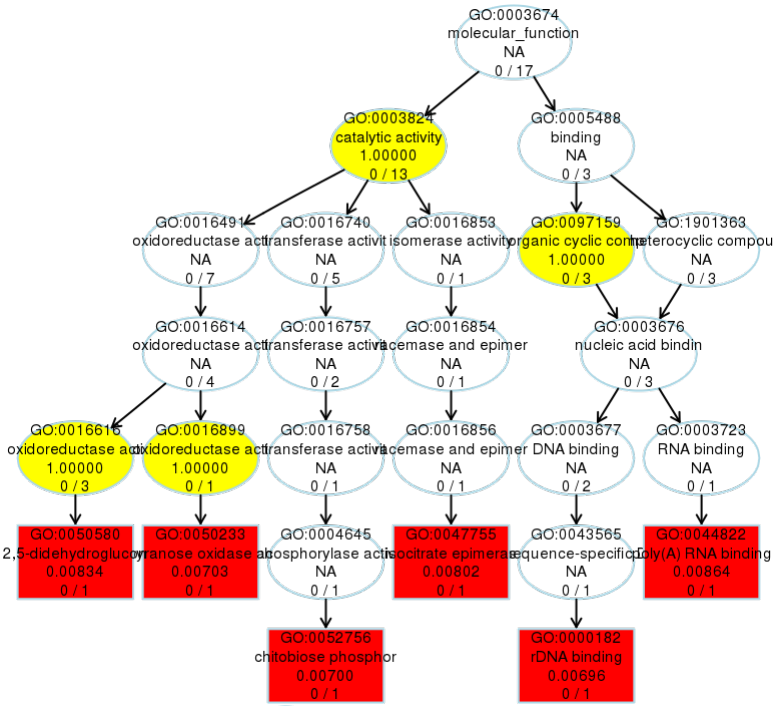
```
## Lets make up some gene lengths
gene_lengths = funkytown[[1]]
gene_lengths$width = sample(nrow(gene_lengths))
gene_lengths$ID = rownames(gene_lengths)
gene_lengths = gene_lengths[,c("ID","width")]

## And some GO categories
goids=funkytown[[1]]
all_go_categories = AnnotationDbi::keys(GO.db)
goids$GO = sample(all_go_categories, nrow(gene_lengths))
goids$ID = rownames(goids)
goids = goids[,c("ID","GO")]

ontology_fun = limma_ontology(funkytown, gene_lengths=gene_lengths, goids=goids, n=100, overwrite=TRUE)
```

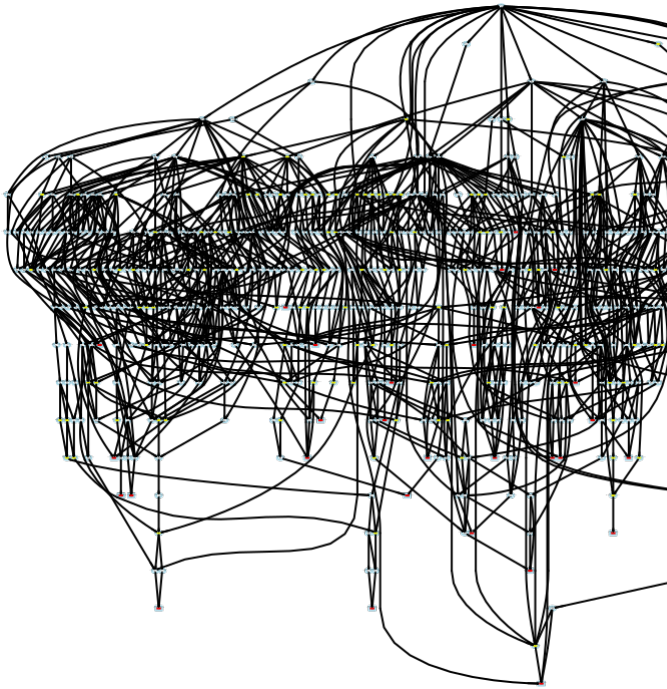
```
## [1] "This function expects a list of limma contrast tables and some annotation information."
## [1] "The annotation information would be gene lengths and ontology ids"
## [1] "simple_goseq() makes some pretty hard assumptions about the data it is fed:"
## [1] "It requires 2 tables, one of GOids which must have columns (gene)ID and GO(category)"
## [1] "The other table is of gene lengths with columns (gene)ID and (gene)width."
## [1] "Other columns are fine, but ignored."

## Using manually entered categories.
```

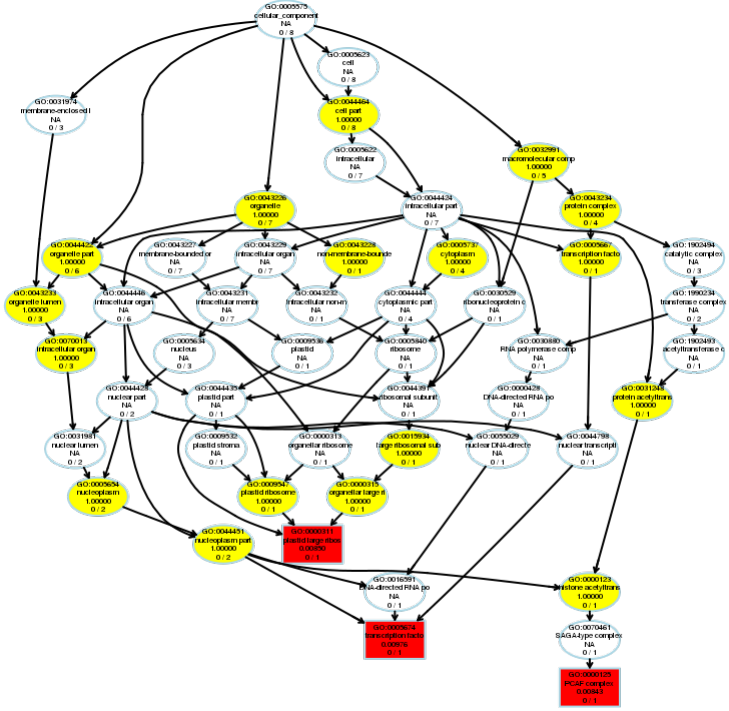
```
## Calculating the p-values...
```



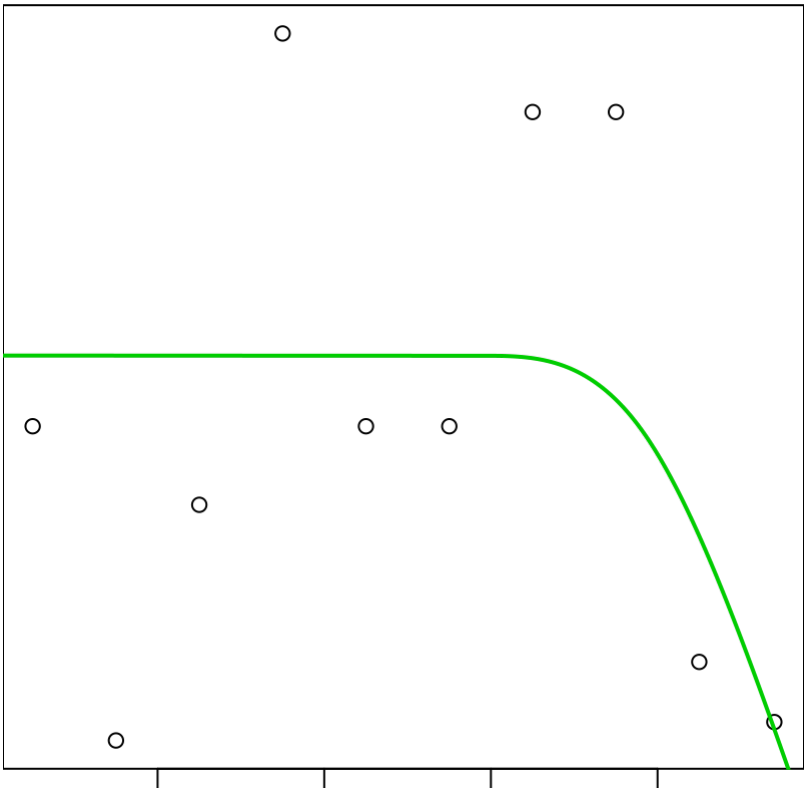

```
## [1] "Calculating q-values"
## [1] "Filling godata table with term information, this takes a while."
## [1] "Making pvalue plots for the ontologies."
## [1] "Attempting to generate a id2go file in the format expected by topGO."
##
## Building most specific GOs ..... ( 17 GO terms found. )
##
## Build GO DAG topology .......... ( 70 GO terms and 76 relations. )
##
## Annotating nodes ............... ( 17 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 75 GO terms found. )
##
## Build GO DAG topology .......... ( 905 GO terms and 1945 relations. )
##
## Annotating nodes ............... ( 75 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 8 GO terms found. )
##
## Build GO DAG topology .......... ( 77 GO terms and 145 relations. )
##
## Annotating nodes ............... ( 8 genes annotated to the GO terms. )
```



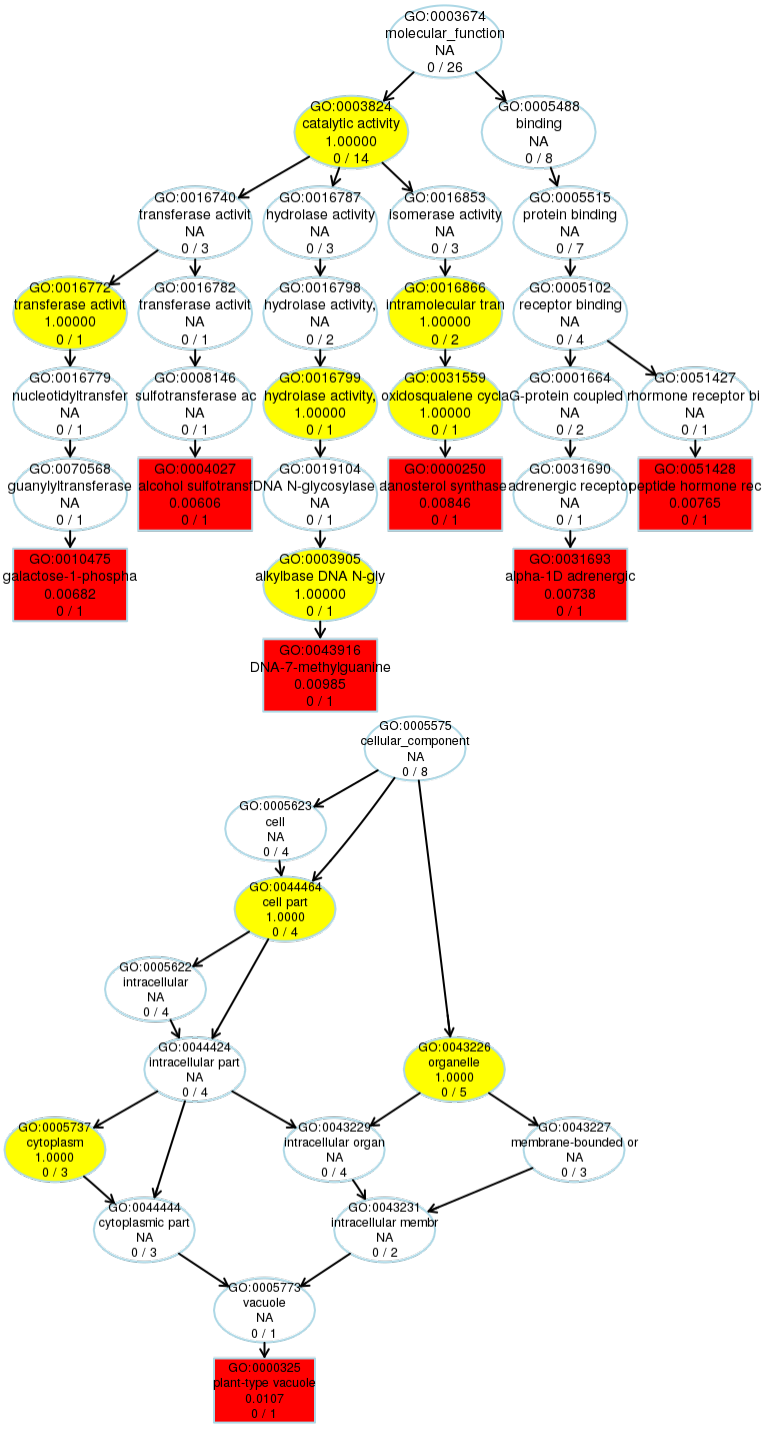


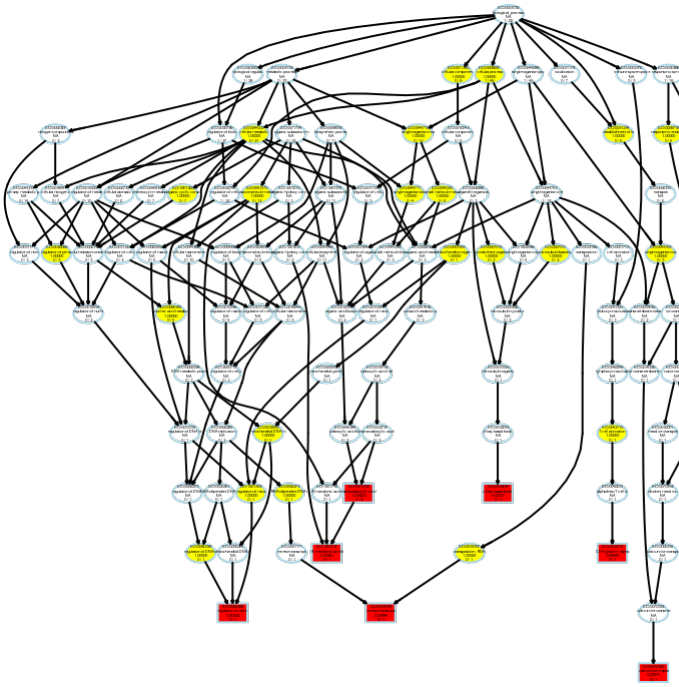


```
## [1] "simple_goseq() makes some pretty hard assumptions about the data it is fed:"
## [1] "It requires 2 tables, one of GOids which must have columns (gene)ID and GO(category)"
## [1] "The other table is of gene lengths with columns (gene)ID and (gene)width."
## [1] "Other columns are fine, but ignored."

## Using manually entered categories.
## Calculating the p-values...
```



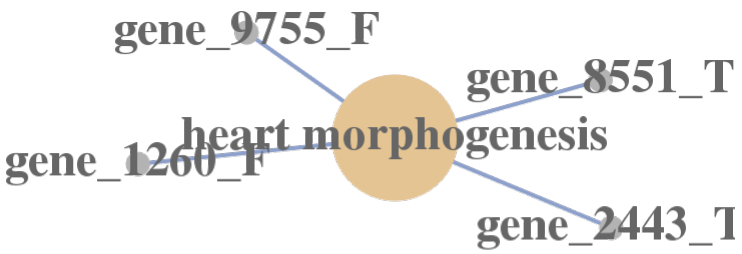
```
## [1] "Calculating q-values"
## [1] "Filling godata table with term information, this takes a while."
## [1] "Making pvalue plots for the ontologies."
##
## Building most specific GOs ..... ( 26 GO terms found. )
##
## Build GO DAG topology .......... ( 102 GO terms and 110 relations. )
##
## Annotating nodes ............... ( 26 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 66 GO terms found. )
##
## Build GO DAG topology .......... ( 758 GO terms and 1545 relations. )
##
## Annotating nodes ............... ( 66 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 8 GO terms found. )
##
## Build GO DAG topology .......... ( 60 GO terms and 95 relations. )
##
## Annotating nodes ............... ( 8 genes annotated to the GO terms. )
```

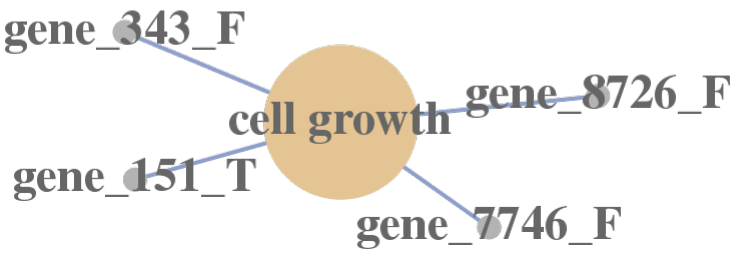




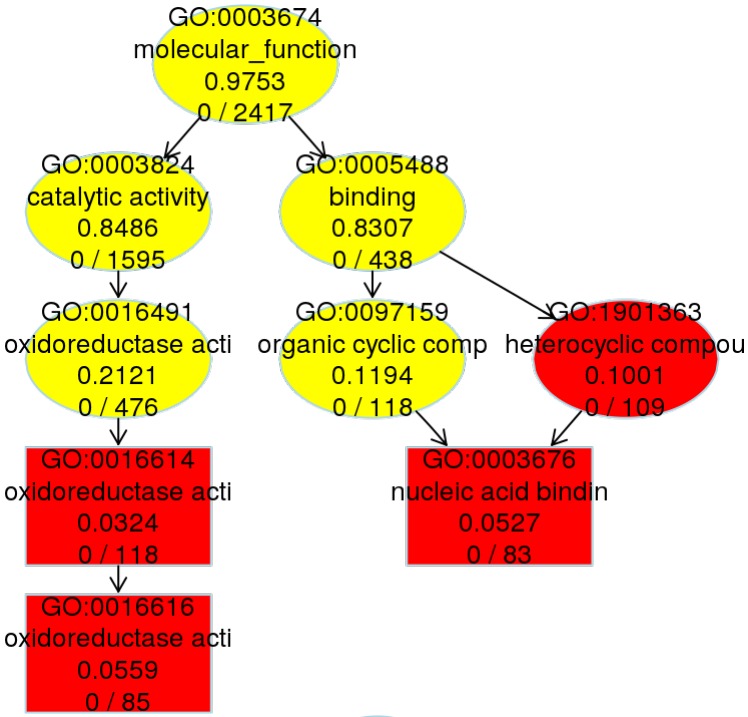
```
## [1] "Using GO mapping data located in GO2EG.rda"
## [1] "Testing gseGO"
## NULL
## [1] "Starting MF(molecular function) analysis"
## The minimum observed adjusted pvalue is: 0.032362
## The minimum observed adjusted pvalue is: 0.186196
## [1] "Starting BP(biological process) analysis"
## The minimum observed adjusted pvalue is: 0.000260
```

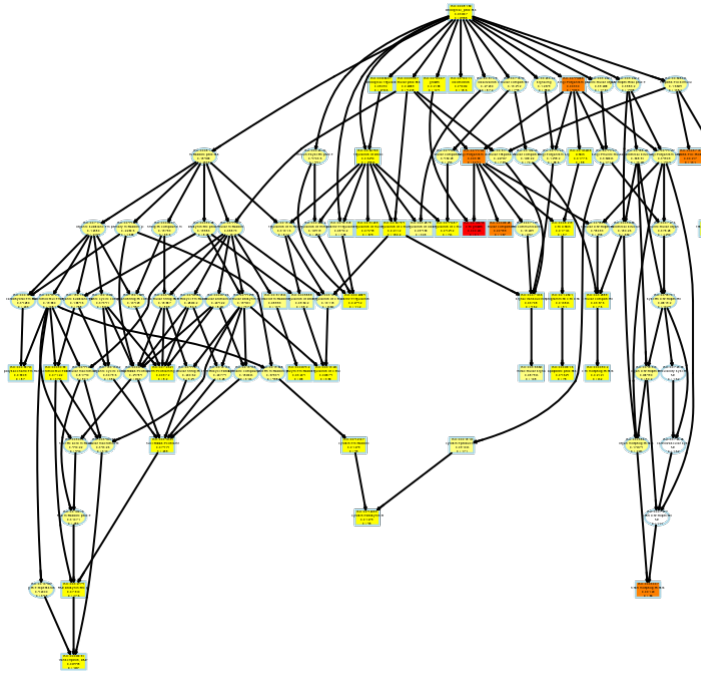
```
## The minimum observed adjusted pvalue is: 0.040515
## [1] "Starting CC(cellular component) analysis"
## The minimum observed adjusted pvalue is: 0.028534
## The minimum observed adjusted pvalue is: 0.172937
## [1] "Attempting to include the cnetplots from clusterProfiler."
## [1] "They fail often, if this is causing errors, set:"
## [1] "include_cnetplots to FALSE"
## [1] "cnetplot just failed for the MF ontology.  Do not be concerned with the previous error."
```

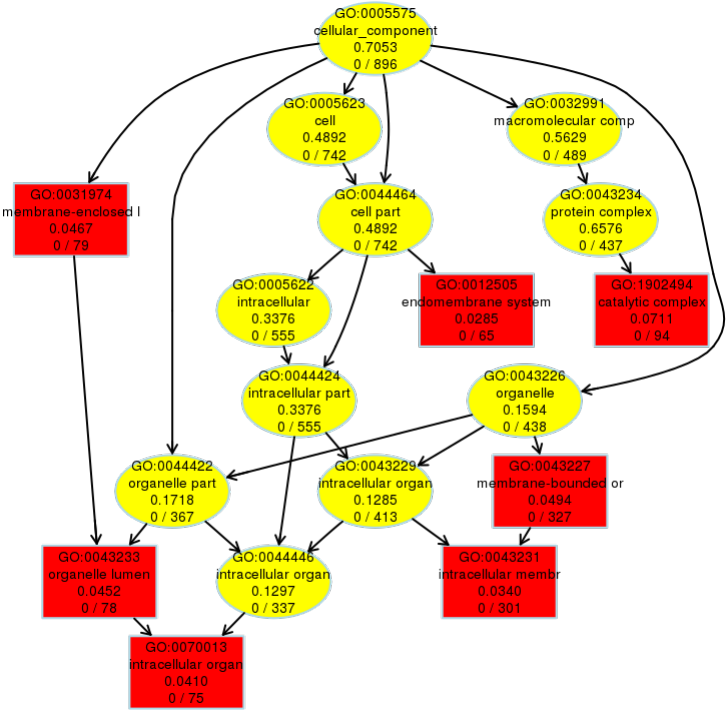





```
## [1] "cnetplot just failed for the CC ontology.  Do not be concerned with the previous error."
##
## Building most specific GOs ..... ( 2417 GO terms found. )
##
## Build GO DAG topology .......... ( 3320 GO terms and 4064 relations. )
##
## Annotating nodes ............... ( 2417 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 6488 GO terms found. )
##
## Build GO DAG topology .......... ( 13785 GO terms and 31277 relations. )
##
## Annotating nodes ............... ( 6488 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 896 GO terms found. )
##
## Build GO DAG topology .......... ( 1454 GO terms and 2752 relations. )
##
## Annotating nodes ............... ( 896 genes annotated to the GO terms. )
```

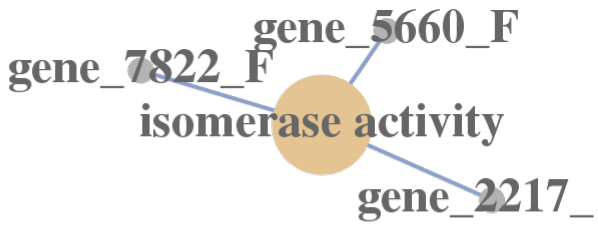


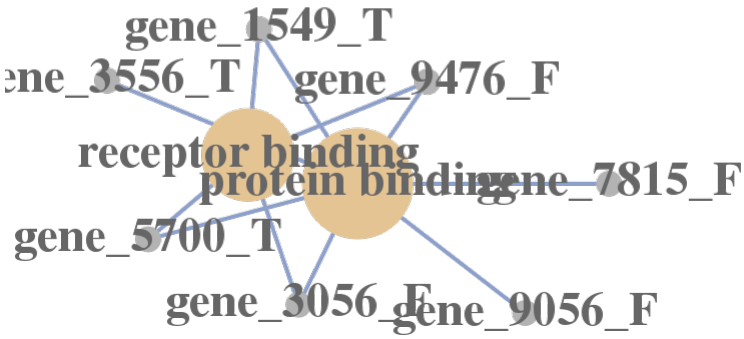




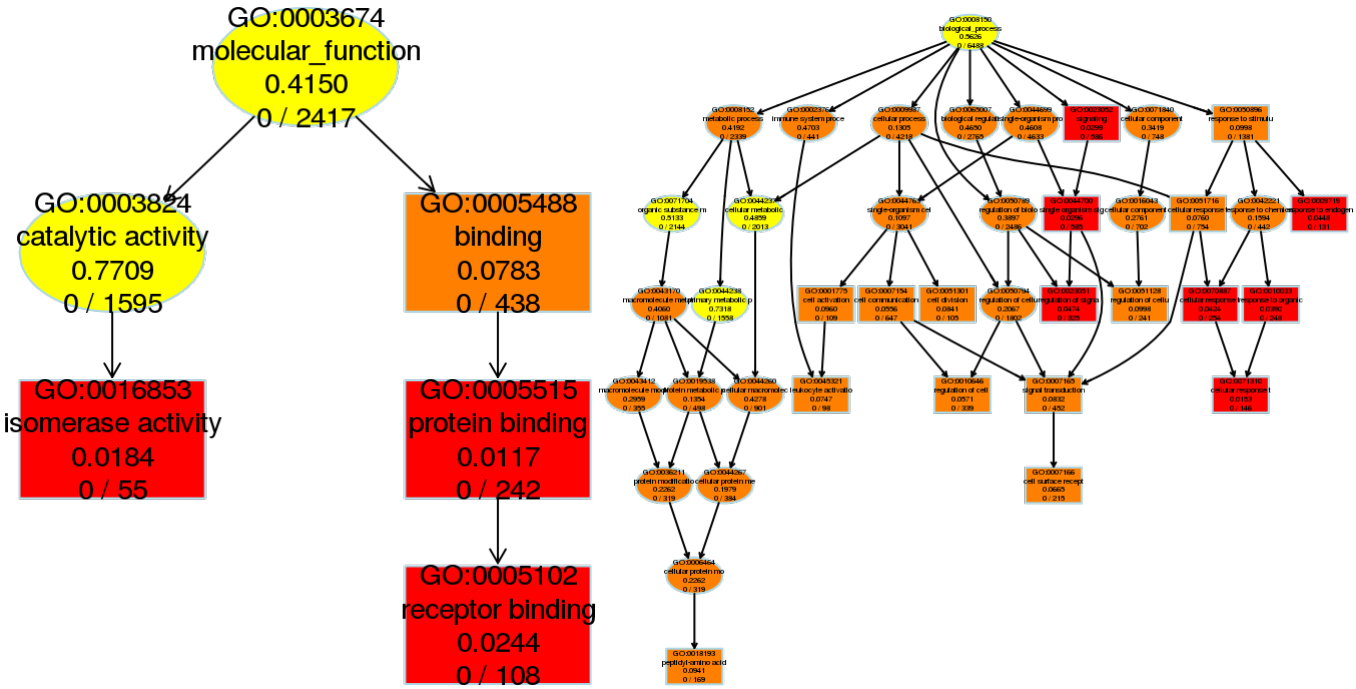

```
## [1] "Using GO mapping data located in GO2EG.rda"
## [1] "Testing gseGO"
## NULL
## [1] "Starting MF(molecular function) analysis"
## The minimum observed adjusted pvalue is: 0.011739
## The minimum observed adjusted pvalue is: 0.073174
## [1] "Starting BP(biological process) analysis"
## The minimum observed adjusted pvalue is: 0.015344
```

```
## The minimum observed adjusted pvalue is: 0.610003
## [1] "Starting CC(cellular component) analysis"
## The minimum observed adjusted pvalue is: 0.464357
## The minimum observed adjusted pvalue is: 0.950772
## [1] "Attempting to include the cnetplots from clusterProfiler."
## [1] "They fail often, if this is causing errors, set:"
## [1] "include_cnetplots to FALSE"
```






```
## [1] "cnetplot just failed for the BP ontology.  Do not be concerned with the previous error."
## [1] "cnetplot just failed for the CC ontology.  Do not be concerned with the previous error."
##
## Building most specific GOs ..... ( 2417 GO terms found. )
##
## Build GO DAG topology .......... ( 3320 GO terms and 4064 relations. )
##
## Annotating nodes ............... ( 2417 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 6488 GO terms found. )
##
## Build GO DAG topology .......... ( 13785 GO terms and 31277 relations. )
##
## Annotating nodes ............... ( 6488 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 896 GO terms found. )
##
## Build GO DAG topology .......... ( 1454 GO terms and 2752 relations. )
##
## Annotating nodes ............... ( 896 genes annotated to the GO terms. )
```




```
##
## Building most specific GOs ..... ( 2417 GO terms found. )
##
## Build GO DAG topology .......... ( 3320 GO terms and 4064 relations. )
##
## Annotating nodes ............... ( 2417 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 6488 GO terms found. )
##
## Build GO DAG topology .......... ( 13785 GO terms and 31277 relations. )
##
## Annotating nodes ............... ( 6488 genes annotated to the GO terms. )
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## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

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## coercion

##
##           -- Classic Algorithm --
```

```
##
##       the algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test

## Warning in .local(object, test.stat, ...): No enrichment can pe performed
## - there are no feasible GO terms!

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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```
## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 3320 nontrivial nodes
##       parameters:
##           test statistic:  KS tests
##           score order:  increasing

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 13785 nontrivial nodes
##       parameters:
##           test statistic:  KS tests
##           score order:  increasing

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 1454 nontrivial nodes
##       parameters:
##           test statistic:  KS tests
##           score order:  increasing

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Elim Algorithm --
##
##       the algorithm is scoring 3320 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test
##           cutOff:  0.01
##           score order:  increasing
##
##   Level 15:  1 nodes to be scored    (0 eliminated genes)
##
##   Level 14:  14 nodes to be scored   (0 eliminated genes)
##
##   Level 13:  21 nodes to be scored   (0 eliminated genes)
##
##   Level 12:  55 nodes to be scored   (0 eliminated genes)
```

```
##
##   Level 11:  73 nodes to be scored   (0 eliminated genes)
##
##   Level 10:  99 nodes to be scored   (0 eliminated genes)
##
##   Level 9:   173 nodes to be scored  (0 eliminated genes)
##
##   Level 8:   322 nodes to be scored  (0 eliminated genes)
##
##   Level 7:   590 nodes to be scored  (0 eliminated genes)
##
##   Level 6:   1068 nodes to be scored (0 eliminated genes)
##
##   Level 5:   543 nodes to be scored  (0 eliminated genes)
##
##   Level 4:   244 nodes to be scored  (0 eliminated genes)
##
##   Level 3:   97 nodes to be scored   (5 eliminated genes)
##
##   Level 2:   19 nodes to be scored   (5 eliminated genes)
##
##   Level 1:   1 nodes to be scored    (5 eliminated genes)

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##          -- Elim Algorithm --
##
##       the algorithm is scoring 13785 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test
##           cutOff:  0.01
##           score order:  increasing
##
##   Level 19:  2 nodes to be scored    (0 eliminated genes)
##
##   Level 18:  10 nodes to be scored   (0 eliminated genes)
##
##   Level 17:  29 nodes to be scored   (0 eliminated genes)
##
##   Level 16:  80 nodes to be scored   (0 eliminated genes)
##
##   Level 15:  205 nodes to be scored  (0 eliminated genes)
##
##   Level 14:  405 nodes to be scored  (0 eliminated genes)
##
##   Level 13:  742 nodes to be scored  (0 eliminated genes)
##
##   Level 12:  1089 nodes to be scored (0 eliminated genes)
##
##   Level 11:  1505 nodes to be scored (0 eliminated genes)
##
##   Level 10:  1881 nodes to be scored (20 eliminated genes)
```

```
##
##   Level 9:   1982 nodes to be scored (27 eliminated genes)
##
##   Level 8:   1921 nodes to be scored (68 eliminated genes)
##
##   Level 7:   1599 nodes to be scored (68 eliminated genes)
##
##   Level 6:   1224 nodes to be scored (68 eliminated genes)
##
##   Level 5:   733 nodes to be scored  (88 eliminated genes)
##
##   Level 4:   289 nodes to be scored  (141 eliminated genes)
##
##   Level 3:   70 nodes to be scored   (149 eliminated genes)
##
##   Level 2:   18 nodes to be scored   (149 eliminated genes)
##
##   Level 1:   1 nodes to be scored    (149 eliminated genes)

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Elim Algorithm --
##
##       the algorithm is scoring 1454 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test
##           cutOff:  0.01
##           score order:  increasing
##
##   Level 19:  1 nodes to be scored    (0 eliminated genes)
##
##   Level 18:  1 nodes to be scored    (0 eliminated genes)
##
##   Level 17:  5 nodes to be scored    (0 eliminated genes)
##
##   Level 16:  11 nodes to be scored   (0 eliminated genes)
##
##   Level 15:  25 nodes to be scored   (0 eliminated genes)
##
##   Level 14:  64 nodes to be scored   (0 eliminated genes)
##
##   Level 13:  83 nodes to be scored   (0 eliminated genes)
##
##   Level 12:  120 nodes to be scored  (0 eliminated genes)
##
##   Level 11:  187 nodes to be scored  (0 eliminated genes)
##
##   Level 10:  177 nodes to be scored  (0 eliminated genes)
##
##   Level 9:   130 nodes to be scored  (0 eliminated genes)
##
##   Level 8:   148 nodes to be scored  (0 eliminated genes)
```

```
##
##   Level 7:   135 nodes to be scored  (0 eliminated genes)
##
##   Level 6:   130 nodes to be scored  (0 eliminated genes)
##
##   Level 5:   104 nodes to be scored  (0 eliminated genes)
##
##   Level 4:   97 nodes to be scored   (0 eliminated genes)
##
##   Level 3:   23 nodes to be scored   (0 eliminated genes)
##
##   Level 2:   12 nodes to be scored   (0 eliminated genes)
##
##   Level 1:   1 nodes to be scored    (0 eliminated genes)

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

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## coercion

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## coercion

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Weight Algorithm --
##
##       The algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test : ratio

## Warning in topGO::getSigGroups(mf_GOdata, test_stat): No enrichment can pe
## performed - there are no feasible GO terms!

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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## coercion

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion
```

```
##
##           -- Weight Algorithm --
##
##       The algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test : ratio

## Warning in topGO::getSigGroups(bp_GOdata, test_stat): No enrichment can pe
## performed - there are no feasible GO terms!

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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## coercion

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Weight Algorithm --
##
##       The algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test : ratio

## Warning in topGO::getSigGroups(cc_GOdata, test_stat): No enrichment can pe
## performed - there are no feasible GO terms!

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

## Warning in data.frame(infoMat, annoStat, apply(l, 2, format.FUN, dig = 2,
## : row names were found from a short variable and have been discarded

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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```

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## Warning in data.frame(infoMat, annoStat, apply(l, 2, format.FUN, dig = 2,
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```

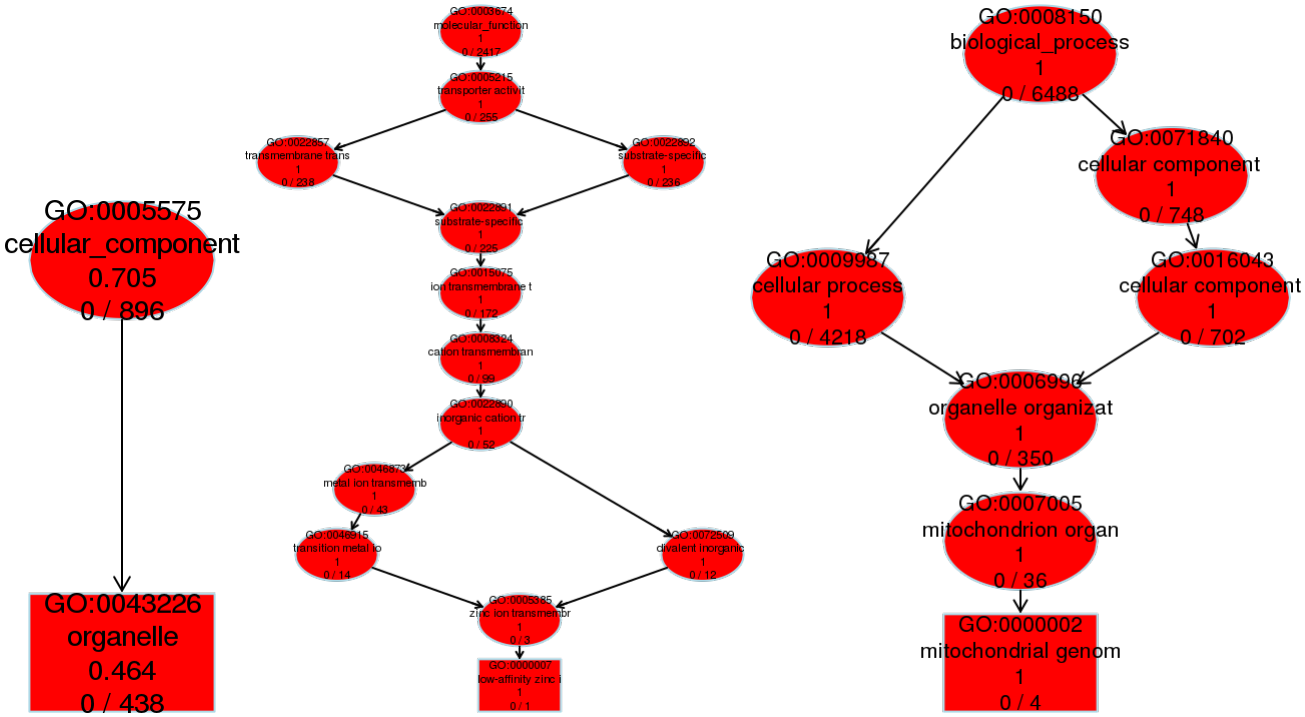


```
##
## Building most specific GOs ..... ( 2417 GO terms found. )
##
## Build GO DAG topology .......... ( 3320 GO terms and 4064 relations. )
##
## Annotating nodes ............... ( 2417 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 6488 GO terms found. )
##
## Build GO DAG topology .......... ( 13785 GO terms and 31277 relations. )
##
## Annotating nodes ............... ( 6488 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 896 GO terms found. )
##
## Build GO DAG topology .......... ( 1454 GO terms and 2752 relations. )
##
## Annotating nodes ............... ( 896 genes annotated to the GO terms. )
```

```
## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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## coercion

##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test

## Warning in .local(object, test.stat, ...): No enrichment can pe performed
## - there are no feasible GO terms!

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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##       the algorithm is scoring 0 nontrivial nodes
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##           test statistic:  Fisher test

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## - there are no feasible GO terms!

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## coercion

##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 3320 nontrivial nodes
##       parameters:
##           test statistic:  KS tests
##           score order:  increasing

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 13785 nontrivial nodes
##       parameters:
##           test statistic:  KS tests
##           score order:  increasing

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 1454 nontrivial nodes
##       parameters:
##           test statistic:  KS tests
##           score order:  increasing

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Elim Algorithm --
```

```
##
##       the algorithm is scoring 3320 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test
##           cutOff:  0.01
##           score order:  increasing
##
##   Level 15:  1 nodes to be scored    (0 eliminated genes)
##
##   Level 14:  14 nodes to be scored   (0 eliminated genes)
##
##   Level 13:  21 nodes to be scored   (0 eliminated genes)
##
##   Level 12:  55 nodes to be scored   (0 eliminated genes)
##
##   Level 11:  73 nodes to be scored   (0 eliminated genes)
##
##   Level 10:  99 nodes to be scored   (0 eliminated genes)
##
##   Level 9:   173 nodes to be scored  (0 eliminated genes)
##
##   Level 8:   322 nodes to be scored  (0 eliminated genes)
##
##   Level 7:   590 nodes to be scored  (0 eliminated genes)
##
##   Level 6:   1068 nodes to be scored (0 eliminated genes)
##
##   Level 5:   543 nodes to be scored  (0 eliminated genes)
##
##   Level 4:   244 nodes to be scored  (0 eliminated genes)
##
##   Level 3:   97 nodes to be scored   (5 eliminated genes)
##
##   Level 2:   19 nodes to be scored   (5 eliminated genes)
##
##   Level 1:   1 nodes to be scored    (5 eliminated genes)

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Elim Algorithm --
##
##       the algorithm is scoring 13785 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test
##           cutOff:  0.01
##           score order:  increasing
##
##   Level 19:  2 nodes to be scored    (0 eliminated genes)
##
##   Level 18:  10 nodes to be scored   (0 eliminated genes)
##
##   Level 17:  29 nodes to be scored   (0 eliminated genes)
```

```
##
##   Level 16:  80 nodes to be scored   (0 eliminated genes)
##
##   Level 15:  205 nodes to be scored  (0 eliminated genes)
##
##   Level 14:  405 nodes to be scored  (0 eliminated genes)
##
##   Level 13:  742 nodes to be scored  (4 eliminated genes)
##
##   Level 12:  1089 nodes to be scored (4 eliminated genes)
##
##   Level 11:  1505 nodes to be scored (4 eliminated genes)
##
##   Level 10:  1881 nodes to be scored (11 eliminated genes)
##
##   Level 9:   1982 nodes to be scored (11 eliminated genes)
##
##   Level 8:   1921 nodes to be scored (15 eliminated genes)
##
##   Level 7:   1599 nodes to be scored (15 eliminated genes)
##
##   Level 6:   1224 nodes to be scored (28 eliminated genes)
##
##   Level 5:   733 nodes to be scored  (48 eliminated genes)
##
##   Level 4:   289 nodes to be scored  (101 eliminated genes)
##
##   Level 3:   70 nodes to be scored   (109 eliminated genes)
##
##   Level 2:   18 nodes to be scored   (109 eliminated genes)
##
##   Level 1:   1 nodes to be scored    (109 eliminated genes)

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Elim Algorithm --
##
##       the algorithm is scoring 1454 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test
##           cutOff:  0.01
##           score order:  increasing
##
##   Level 19:  1 nodes to be scored    (0 eliminated genes)
##
##   Level 18:  1 nodes to be scored    (0 eliminated genes)
##
##   Level 17:  5 nodes to be scored    (0 eliminated genes)
##
##   Level 16:  11 nodes to be scored   (0 eliminated genes)
##
##   Level 15:  25 nodes to be scored   (0 eliminated genes)
```

```
##
##   Level 14:  64 nodes to be scored   (0 eliminated genes)
##
##   Level 13:  83 nodes to be scored   (0 eliminated genes)
##
##   Level 12:  120 nodes to be scored  (0 eliminated genes)
##
##   Level 11:  187 nodes to be scored  (0 eliminated genes)
##
##   Level 10:  177 nodes to be scored  (0 eliminated genes)
##
##   Level 9:   130 nodes to be scored  (0 eliminated genes)
##
##   Level 8:   148 nodes to be scored  (0 eliminated genes)
##
##   Level 7:   135 nodes to be scored  (0 eliminated genes)
##
##   Level 6:   130 nodes to be scored  (0 eliminated genes)
##
##   Level 5:   104 nodes to be scored  (0 eliminated genes)
##
##   Level 4:   97 nodes to be scored   (0 eliminated genes)
##
##   Level 3:   23 nodes to be scored   (0 eliminated genes)
##
##   Level 2:   12 nodes to be scored   (0 eliminated genes)
##
##   Level 1:   1 nodes to be scored    (0 eliminated genes)

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

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##
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##
##       The algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test : ratio

## Warning in topGO::getSigGroups(mf_GOdata, test_stat): No enrichment can pe
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## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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##
##           -- Weight Algorithm --
##
##       The algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test : ratio

## Warning in topGO::getSigGroups(bp_GOdata, test_stat): No enrichment can pe
## performed - there are no feasible GO terms!

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## coercion

##
##           -- Weight Algorithm --
##
##       The algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test : ratio

## Warning in topGO::getSigGroups(cc_GOdata, test_stat): No enrichment can pe
## performed - there are no feasible GO terms!

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

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```

```
## Warning in data.frame(infoMat, annoStat, apply(l, 2, format.FUN, dig = 2,
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## : row names were found from a short variable and have been discarded
```
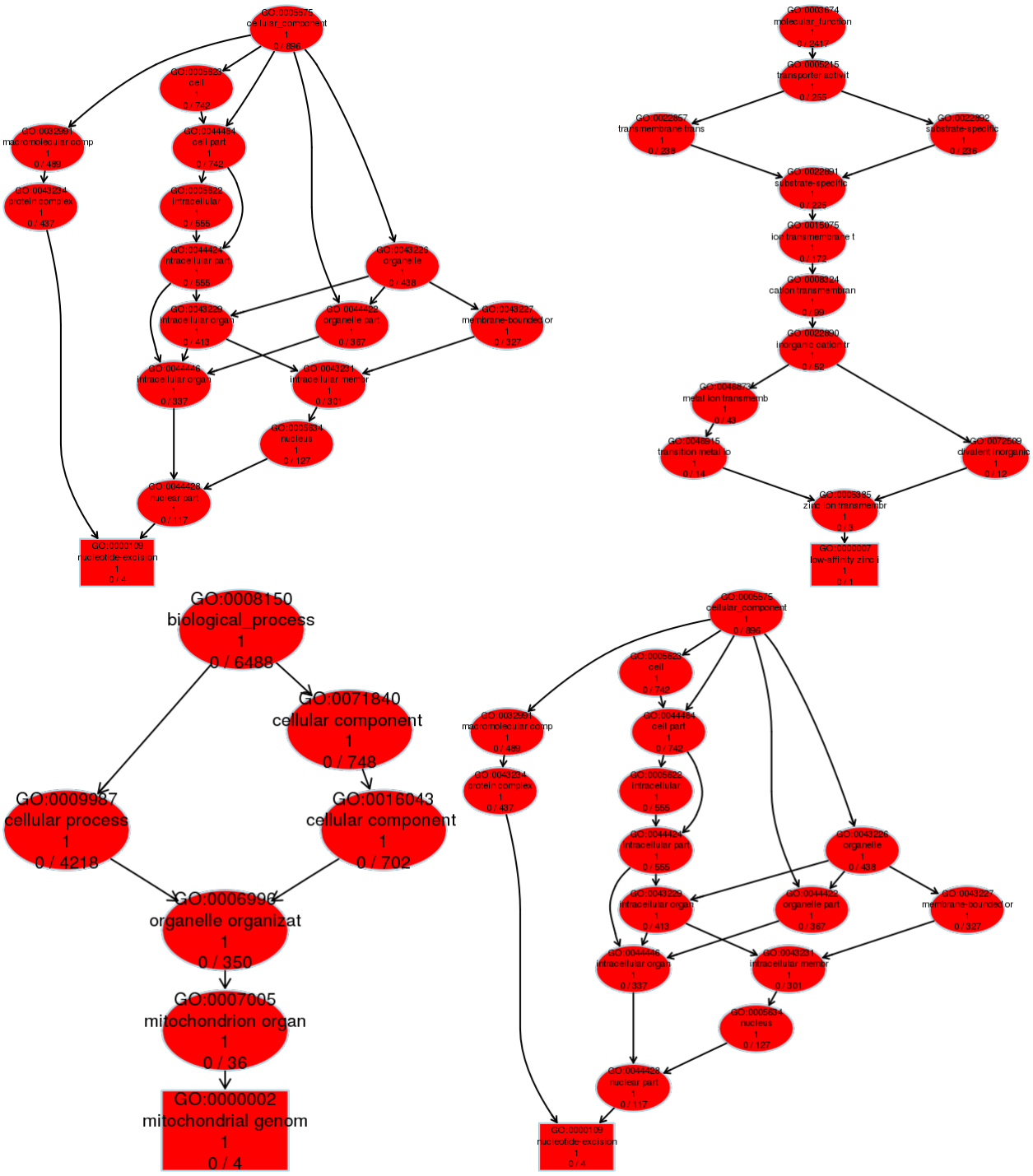



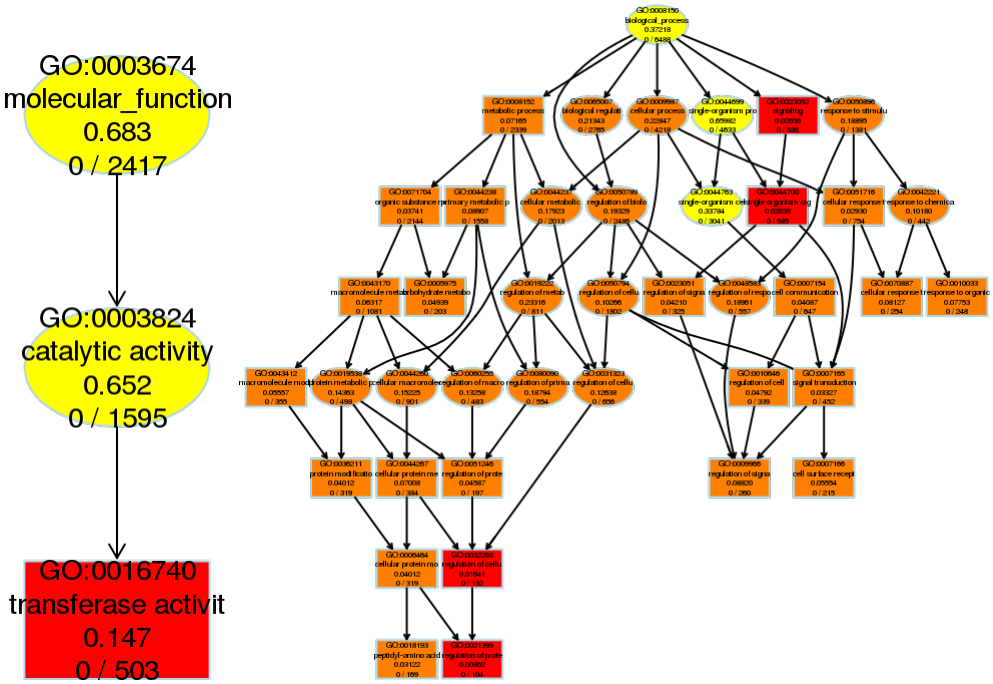

```
testme = head(funkytown[[1]], n=40)
tt = simple_clusterprofiler(testme, goids=goids, gff=goids)
```

```
## [1] "Using GO mapping data located in GO2EG.rda"
## [1] "Testing gseGO"
## NULL
## [1] "Starting MF(molecular function) analysis"
## The minimum observed adjusted pvalue is: 0.147395
```

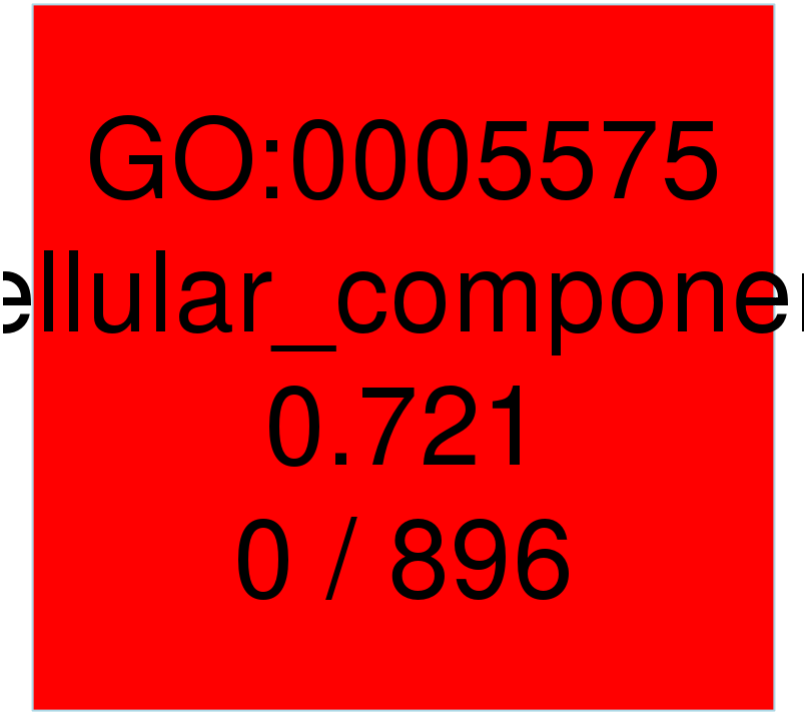
```
## The minimum observed adjusted pvalue is: 0.442185
## [1] "Starting BP(biological process) analysis"
## The minimum observed adjusted pvalue is: 0.008624
## The minimum observed adjusted pvalue is: 0.202672
## [1] "Starting CC(cellular component) analysis"
## The minimum observed adjusted pvalue is: 0.721347
## The minimum observed adjusted pvalue is: 0.721347
## [1] "Attempting to include the cnetplots from clusterProfiler."
## [1] "They fail often, if this is causing errors, set:"
## [1] "include_cnetplots to FALSE"
## [1] "cnetplot just failed for the MF ontology.  Do not be concerned with the previous error."
## [1] "cnetplot just failed for the BP ontology.  Do not be concerned with the previous error."
## [1] "cnetplot just failed for the CC ontology.  Do not be concerned with the previous error."
```

```
ttt = cluster_trees(testme, tt)
```

```
##
## Building most specific GOs ..... ( 2417 GO terms found. )
##
## Build GO DAG topology .......... ( 3320 GO terms and 4064 relations. )
##
## Annotating nodes ............... ( 2417 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 6488 GO terms found. )
##
## Build GO DAG topology .......... ( 13785 GO terms and 31277 relations. )
##
## Annotating nodes ............... ( 6488 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 896 GO terms found. )
##
## Build GO DAG topology .......... ( 1454 GO terms and 2752 relations. )
##
## Annotating nodes ............... ( 896 genes annotated to the GO terms. )
```




```
## Nothing to do:
```




```
tttt = simple_topgo(testme)
```

```
##
## Building most specific GOs ..... ( 2417 GO terms found. )
##
```

```
## Build GO DAG topology .......... ( 3320 GO terms and 4064 relations. )
##
## Annotating nodes ............... ( 2417 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 6488 GO terms found. )
##
## Build GO DAG topology .......... ( 13785 GO terms and 31277 relations. )
##
## Annotating nodes ............... ( 6488 genes annotated to the GO terms. )
##
## Building most specific GOs ..... ( 896 GO terms found. )
##
## Build GO DAG topology .......... ( 1454 GO terms and 2752 relations. )
##
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## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test

## Warning in .local(object, test.stat, ...): No enrichment can pe performed
## - there are no feasible GO terms!

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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## - there are no feasible GO terms!

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 3320 nontrivial nodes
##       parameters:
##           test statistic:  KS tests
##           score order:  increasing

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 13785 nontrivial nodes
##       parameters:
##           test statistic:  KS tests
##           score order:  increasing

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion
```

```
##
##           -- Classic Algorithm --
##
##       the algorithm is scoring 1454 nontrivial nodes
##       parameters:
##           test statistic:  KS tests
##           score order:  increasing

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Elim Algorithm --
##
##       the algorithm is scoring 3320 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test
##           cutOff:  0.01
##           score order:  increasing
##
##   Level 15:  1 nodes to be scored    (0 eliminated genes)
##
##   Level 14:  14 nodes to be scored   (0 eliminated genes)
##
##   Level 13:  21 nodes to be scored   (0 eliminated genes)
##
##   Level 12:  55 nodes to be scored   (0 eliminated genes)
##
##   Level 11:  73 nodes to be scored   (0 eliminated genes)
##
##   Level 10:  99 nodes to be scored   (0 eliminated genes)
##
##   Level 9:   173 nodes to be scored  (0 eliminated genes)
##
##   Level 8:   322 nodes to be scored  (0 eliminated genes)
##
##   Level 7:   590 nodes to be scored  (0 eliminated genes)
##
##   Level 6:   1068 nodes to be scored (0 eliminated genes)
##
##   Level 5:   543 nodes to be scored  (0 eliminated genes)
##
##   Level 4:   244 nodes to be scored  (0 eliminated genes)
##
##   Level 3:   97 nodes to be scored   (5 eliminated genes)
##
##   Level 2:   19 nodes to be scored   (5 eliminated genes)
##
##   Level 1:   1 nodes to be scored    (5 eliminated genes)

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
```

```
##           -- Elim Algorithm --
##
##       the algorithm is scoring 13785 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test
##           cutOff:  0.01
##           score order:  increasing
##
##   Level 19:  2 nodes to be scored    (0 eliminated genes)
##
##   Level 18:  10 nodes to be scored   (0 eliminated genes)
##
##   Level 17:  29 nodes to be scored   (0 eliminated genes)
##
##   Level 16:  80 nodes to be scored   (0 eliminated genes)
##
##   Level 15:  205 nodes to be scored  (0 eliminated genes)
##
##   Level 14:  405 nodes to be scored  (0 eliminated genes)
##
##   Level 13:  742 nodes to be scored  (0 eliminated genes)
##
##   Level 12:  1089 nodes to be scored (0 eliminated genes)
##
##   Level 11:  1505 nodes to be scored (0 eliminated genes)
##
##   Level 10:  1881 nodes to be scored (20 eliminated genes)
##
##   Level 9:   1982 nodes to be scored (27 eliminated genes)
##
##   Level 8:   1921 nodes to be scored (68 eliminated genes)
##
##   Level 7:   1599 nodes to be scored (68 eliminated genes)
##
##   Level 6:   1224 nodes to be scored (68 eliminated genes)
##
##   Level 5:   733 nodes to be scored  (88 eliminated genes)
##
##   Level 4:   289 nodes to be scored  (104 eliminated genes)
##
##   Level 3:   70 nodes to be scored   (112 eliminated genes)
##
##   Level 2:   18 nodes to be scored   (112 eliminated genes)
##
##   Level 1:   1 nodes to be scored    (112 eliminated genes)

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
## coercion

##
##           -- Elim Algorithm --
##
##       the algorithm is scoring 1454 nontrivial nodes
##       parameters:
```

```
##           test statistic:  Fisher test
##           cutOff:  0.01
##           score order:  increasing
##
##   Level 19:  1 nodes to be scored    (0 eliminated genes)
##
##   Level 18:  1 nodes to be scored    (0 eliminated genes)
##
##   Level 17:  5 nodes to be scored    (0 eliminated genes)
##
##   Level 16:  11 nodes to be scored   (0 eliminated genes)
##
##   Level 15:  25 nodes to be scored   (0 eliminated genes)
##
##   Level 14:  64 nodes to be scored   (0 eliminated genes)
##
##   Level 13:  83 nodes to be scored   (0 eliminated genes)
##
##   Level 12:  120 nodes to be scored  (0 eliminated genes)
##
##   Level 11:  187 nodes to be scored  (0 eliminated genes)
##
##   Level 10:  177 nodes to be scored  (0 eliminated genes)
##
##   Level 9:   130 nodes to be scored  (0 eliminated genes)
##
##   Level 8:   148 nodes to be scored  (0 eliminated genes)
##
##   Level 7:   135 nodes to be scored  (0 eliminated genes)
##
##   Level 6:   130 nodes to be scored  (0 eliminated genes)
##
##   Level 5:   104 nodes to be scored  (0 eliminated genes)
##
##   Level 4:   97 nodes to be scored   (0 eliminated genes)
##
##   Level 3:   23 nodes to be scored   (0 eliminated genes)
##
##   Level 2:   12 nodes to be scored   (0 eliminated genes)
##
##   Level 1:   1 nodes to be scored    (0 eliminated genes)

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## coercion
```

```
##
##           -- Weight Algorithm --
##
##       The algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test : ratio

## Warning in topGO::getSigGroups(mf_GOdata, test_stat): No enrichment can pe
## performed - there are no feasible GO terms!

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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##
##           -- Weight Algorithm --
##
##       The algorithm is scoring 0 nontrivial nodes
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## Warning in topGO::getSigGroups(bp_GOdata, test_stat): No enrichment can pe
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## coercion

##
##           -- Weight Algorithm --
##
##       The algorithm is scoring 0 nontrivial nodes
##       parameters:
##           test statistic:  Fisher test : ratio
```

```
## Warning in topGO::getSigGroups(cc_GOdata, test_stat): No enrichment can pe
## performed - there are no feasible GO terms!

## Warning in object@geneSelectionFun(object@allScores): NAs introduced by
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## : row names were found from a short variable and have been discarded
```

A cell-means model using all conditions and batches

```
## acb stands for "kept_conditions_batches"  which takes too long to
## type when setting up the contrasts.
acb = paste0(kept_qcpml2$conditions, kept_qcpml2$batches)
kept_data = exprs(kept_qcpml2$expressionset)
table(acb)
## The invocation of table() keptows me to count up the contribution of
## each condition/batch combination to the whole data set.

## Doing this (as I understand it) means I do nothave to worry about
## balanced samples so much, but must be more careful to understand
## the relative contribution of each sample type to the entire data
## set.

complete_model = model.matrix(~0 + acb)
```

```
complete_fit = lmFit(kept_data, complete_model)
complete_voom = my_voom(kept_data, complete_model)
complete_voom$plot
complete_model
## This is an example of what happens when I have heterogenous numbers of samples
## on each side of a contrast, so that a normal design matrix of conditions + batches
## would not work, so instead I add up the contributions of each batch (capital letters)
## and average them out, then use the resulting terms in the various contrasts below.
epi_cl14 = "acbcl14_epiF"
epi_clbr = "acbclbr_epiE"
tryp_cl14 = "(acbcl14_trypB + acbcl14_trypD + acbcl14_trypG) / 3"
tryp_clbr = "acbclbr_trypG"
a60_cl14 =  "(acbcl14_a60A * 2/3) + (acbcl14_a60B * 1/3)"
a60_clbr = "acbclbr_a60A"
a96_cl14 = "acbcl14_a96C"
a96_clbr = "acbclbr_a96C"
epi_cl14clbr = paste0("(",epi_cl14,")", "  -  ", "(",epi_clbr,")")
tryp_cl14clbr = paste0("(",tryp_cl14,")", "  -  ", "(",tryp_clbr,")")
a60_cl14clbr = paste0("(",a60_cl14,")", "  -  ", "(",a60_clbr,")")
a96_cl14clbr = paste0("(",a96_cl14,")", "  -  ", "(",a96_clbr,")")
epitryp_cl14 = paste0("(",tryp_cl14,")", "  -  ", "(",epi_cl14,")")
epitryp_clbr = paste0("(",tryp_clbr,")", "  -  ", "(",epi_clbr,")")
epia60_cl14 = paste0("(",a60_cl14,")", "  -  ", "(",epi_cl14,")")
epia60_clbr = paste0("(",a60_clbr,")", "  -  ", "(",epi_clbr,")")
a60a96_cl14 = paste0("(",a96_cl14,")", "  -  ", "(",a60_cl14,")")
a60a96_clbr = paste0("(",a96_clbr,")", "  -  ", "(",a60_clbr,")")
a60tryp_cl14 = paste0("(",tryp_cl14,")", "  -  ", "(",a60_cl14,")")
a60tryp_clbr = paste0("(",tryp_clbr,")", "  -  ", "(",a60_clbr,")")
## The following contrast is messed up in some as of yet unknown way.
epitryp_cl14clbr = paste0("(",epitryp_cl14,")", "  -  ", "(",epitryp_clbr,")")
## So I will add some more contrasts using data which doesn't get screwed up
epia60_cl14clbr = paste0("(",epia60_cl14,")", "  -  ", "(",epia60_clbr,")")
a60tryp_cl14clbr = paste0("(",a60tryp_cl14,")", "  -  ", "(",a60tryp_clbr,")")
a60a96_cl14clbr = paste0("(",a60a96_cl14,")", "  -  ", "(",a60a96_clbr,")")

complete_contrasts_v2 = makeContrasts(
    epi_cl14=epi_cl14,
    epi_clbr=epi_clbr,
    tryp_cl14=tryp_cl14,
    tryp_clbr=tryp_clbr,
    a60_cl14=a60_cl14,
    a60_clbr=a60_clbr,
    a96_cl14=a96_cl14,
    a96_clbr=a96_clbr,
    epi_cl14clbr=epi_cl14clbr,
    tryp_cl14clbr=tryp_cl14clbr,
    a60_cl14clbr=a60_cl14clbr,
    a96_cl14clbr=a96_cl14clbr,
    epitryp_cl14=epitryp_cl14,
    epitryp_clbr=epitryp_clbr,
    epia60_cl14=epia60_cl14,
    epia60_clbr=epia60_clbr,
    a60a96_cl14=a60a96_cl14,
```

```
    a60a96_clbr=a60a96_clbr,
    a60tryp_cl14=a60tryp_cl14,
    a60tryp_clbr=a60tryp_clbr,
    epitryp_cl14clbr=epitryp_cl14clbr,
    epia60_cl14clbr=epia60_cl14clbr,
    a60tryp_cl14clbr=a60tryp_cl14clbr,
    a60a96_cl14clbr=a60a96_cl14clbr,
    levels=complete_voom$design)
## This colnames() is annoyingly necessary to avoid really obnoxious contrast names.
colnames(complete_contrasts_v2) = c("epi_cl14","epi_clbr","tryp_cl14","tryp_clbr","a60_cl14","a60_clbr"
kept_fits = contrasts.fit(complete_fit, complete_contrasts_v2)
kept_comparisons = eBayes(kept_fits)
```

Clean conditions, batches

On the other hand, I would like to perform arbitrary comparisons among my data even when the batches and conditions look good, so I set up my model/contrast matrices a little strangely even then:

```
all_data = exprs(norm_expt$expressionset)
complete_model = model.matrix(~0 + all_human_expt$conditions + all_human_expt$batches)
## Shorten the column names of the model so I don't have to type so much later...
tmpnames = colnames(complete_model)
tmpnames = gsub("all_human_expt[[:punct:]]","", tmpnames)
tmpnames = gsub("conditions","", tmpnames)
colnames(complete_model) = tmpnames
rm(tmpnames)

complete_voom = my_voom(all_data, complete_model)
complete_voom$plot
complete_fit = lmFit(complete_voom, complete_model)

all_contrasts = makeContrasts(
    ## Start with the simple coefficient groupings for each condition
    none4=none4,
    none24=none24,
    none48=none48,
    none72=none72,
    bead4=bead4,
    bead24=bead24,
    bead48=bead48,
    bead72=bead72,
    maj4=maj4,
    maj24=maj24,
    maj48=maj48,
    maj72=maj72,
    ama4=ama4,
    ama24=ama24,
    ama48=ama48,
    ama72=ama72,
    ## Now do a few simple comparisons
    ## compare beads to uninfected
    beadnone_4=bead4-none4,
    beadnone_24=bead24-none24,
```

```
    beadnone_48=bead48-none48,
    beadnone_72=bead72-none72,
    majnone_4=maj4-none4,
    majnone_24=maj24-none24,
    majnone_48=maj48-none48,
    majnone_72=maj72-none72,
    amanone_4=ama4-none4,
    amanone_24=ama24-none24,
    amanone_48=ama48-none48,
    amanone_72=ama72-none72,
    ## compare samples to beads
    majbead_4=maj4-bead4,
    majbead_24=maj24-bead24,
    majbead_48=maj48-bead48,
    majbead_72=maj72-bead72,
    amabead_4=ama4-bead4,
    amabead_24=ama24-bead24,
    amabead_48=ama48-bead48,
    amabead_72=ama72-bead72,
    ## (x-z)-(a-b)
    ## Use this to compare major and amazonensis
    amamaj_bead_4=(ama4-bead4)-(maj4-bead4),
    amamaj_bead_24=(ama24-bead24)-(maj24-bead24),
    amamaj_bead_48=(ama48-bead48)-(maj48-bead48),
    amamaj_bead_72=(ama72-bead72)-(maj72-bead72),
    ## (c-d)-(e-f) where c/d are: (amazon|major/none)/(beads/none)
    majbead_none_4=(maj4-none4)-(bead4-none4),
    majbead_none_24=(maj24-none24)-(bead24-none24),
    majbead_none_48=(maj48-none48)-(bead48-none48),
    majbead_none_72=(maj72-none72)-(bead72-none72),
    amabead_none_4=(ama4-none4)-(bead4-none4),
    amabead_none_24=(ama24-none24)-(bead24-none24),
    amabead_none_48=(ama48-none48)-(bead48-none48),
    amabead_none_72=(ama72-none72)-(bead72-none72),
    levels=complete_voom$design)
all_fits = contrasts.fit(complete_fit, all_contrasts)
all_comparisons = eBayes(all_fits)
limma_list = write_limma(data=all_comparisons)

all_table = topTable(all_comparisons, adjust="fdr", n=nrow(all_data))
write.csv(all_comparisons, file="excel/all_tables.csv")
## write_limma() is a shortcut for writing out all the data structures
all_comparison_tables = write_limma(all_comparisons, excel=FALSE)
```

Ontology searches

The following is an example of a simplified GO search given 20 groups of genes which are from an unannotated organism, but for which blast2GO was performed.

```
ontology_info = read.csv(file="data/trinotate_go_trimmed.csv.gz", header=FALSE, sep="\t")
colnames(ontology_info) = c("gene_id","transcript_id","group","startend","blast_go","pfam_go")
## Drop any entries which don't have a putative length
ontology_info = subset(ontology_info, startend != 0)
```

```
## Split the column 'startend' into two columns by the '-' sign
ontology_info = as.data.frame(transform(ontology_info, startend=colsplit(startend, split="\\-", names=c
## Make the resulting pieces into two separate columns, start and end.
ontology_info$start = ontology_info$startend$start
ontology_info$end = ontology_info$startend$end
## Use start and end to make length
ontology_info$length = abs(ontology_info$start - ontology_info$end)
## Drop the unneeded columns
ontology_info = ontology_info[,c("gene_id","transcript_id","group","start","end","length","blast_go","p
head(ontology_info)
```

```
##    gene_id transcript_id group start  end length
## 1  c111_g1    c111_g1_i1     1   833 2068   1235
## 2  c753_g1    c753_g1_i1     1    50 1660   1610
## 3  c777_g1    c777_g1_i1     1  1433 3670   2237
## 4  c777_g1    c777_g1_i2     1  1553 3790   2237
## 5 c1076_g1   c1076_g1_i1     1   133 2601   2468
## 6 c2482_g1   c2482_g1_i2     1  1112 1612    500
##
## 1
## 2 GO:0031225 GO:0046658 GO:0048046 GO:0005618 GO:0016020 GO:0009505 GO:0005886 GO:0009506 GO:0005774
## 3                                                                                GO:0016021 GO:0005886
## 4                                                                                GO:0016021 GO:0005886
## 5                                                                                GO:0030176 GO:0008219
## 6
##      pfam_go
## 1          0
## 2
## 3
## 4
## 5          0
## 6 GO:0005515
```

```
## goseq() requires mappings between ID/length and ID/GO category
## Currently I have my toy set to assume column names, which is admittedly stupid.
gene_lengths = ontology_info[,c("transcript_id","length")]
colnames(gene_lengths) = c("ID","width")
split_go = ontology_info[,c("transcript_id","blast_go")]
split_go$blast_go = as.character(split_go$blast_go)

## The following few lines were pulled from the internet
## they serve to generate a data structure in the format expected by goseq()
## It simply splits all space separated GO categories into separate rows
## with the same ID
require.auto("splitstackshape")
```

```
## [1] "Loading splitstackshape"
```

```
id_go = concat.split.multiple(split_go, "blast_go", seps=" ", "long")
```

```
## This function is deprecated. Use `cSplit` instead.
```

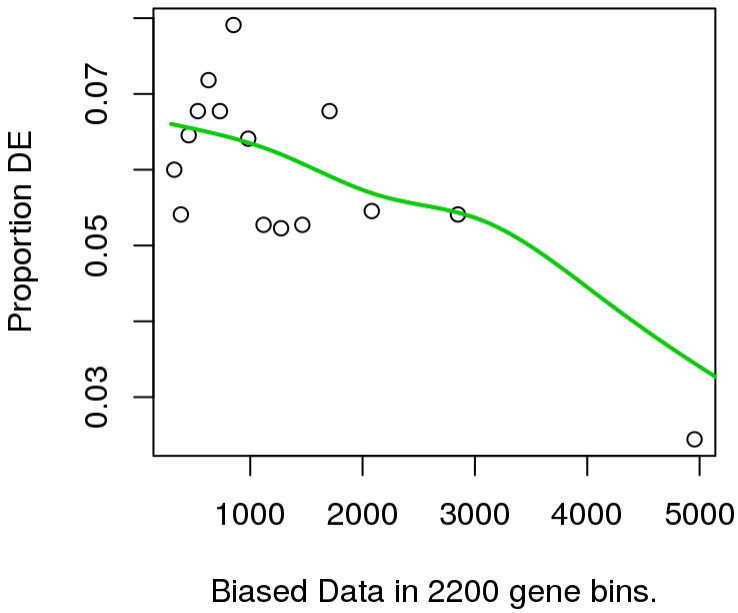
```
id_go = as.data.frame(id_go)
colnames(id_go) = c("ID","GO")
go_ids = subset(id_go, GO != 0)

## Pull out all entries from group 1
group_one = subset(ontology_info, group == "1")
group_one = group_one[,c("transcript_id","start","end")]
colnames(group_one) = c("ID","start","end")

## Perform the goseq() analysis
group_one_go = simple_goseq(group_one, lengths=gene_lengths, goids=go_ids)
```

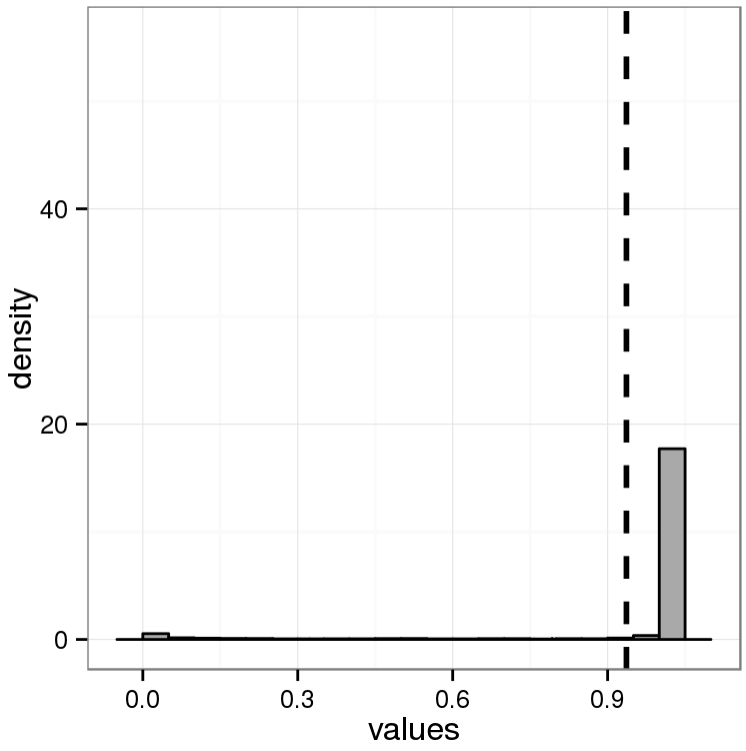
```
## [1] "simple_goseq() makes some pretty hard assumptions about the data it is fed:"
## [1] "It requires 2 tables, one of GOids which must have columns (gene)ID and GO(category)"
## [1] "The other table is of gene lengths with columns (gene)ID and (gene)width."
## [1] "Other columns are fine, but ignored."

## Using manually entered categories.
## Calculating the p-values...
```




```
## [1] "Calculating q-values"
## [1] "Filling godata table with term information, this takes a while."
## [1] "Making pvalue plots for the ontologies."
```

```
group_one_go$pvalue_histogram
```




```
head(group_one_go$godata_interesting)
```

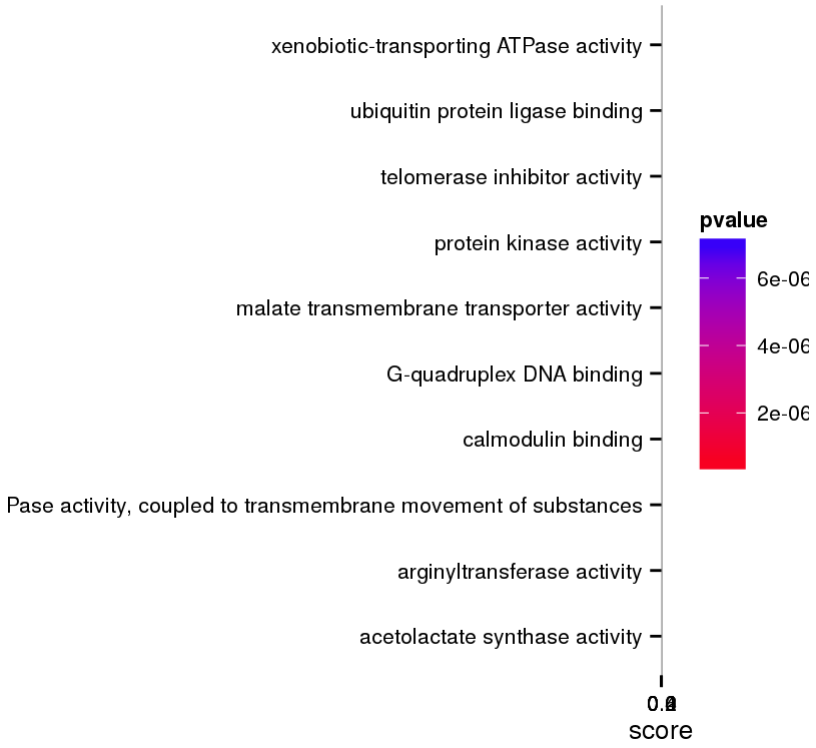
```
##        category over_represented_pvalue under_represented_pvalue
## 2415 GO:0008911            4.151763e-13                         1
## 714  GO:0004057            2.421000e-12                         1
## 3664 GO:0016598            2.421000e-12                         1
## 6529 GO:0050994            2.421000e-12                         1
## 4931 GO:0034077            1.485178e-10                         1
## 5763 GO:0045151            1.485178e-10                         1
##      numDEInCat numInCat       qvalue ont
## 2415         10       10 3.264946e-09  MF
## 714          12       16 4.759685e-09  MF
## 3664         12       16 4.759685e-09  BP
## 6529         12       16 4.759685e-09  BP
## 4931          8        8 1.946573e-07  BP
## 5763          8        8 1.946573e-07  BP
##                                      term
## 2415   lactaldehyde dehydrogenase activity
## 714            arginyltransferase activity
## 3664                 protein arginylation
## 6529 regulation of lipid catabolic process
## 4931          butanediol metabolic process
## 5763          acetoin biosynthetic process
```

```
head(group_one_go$mf_subset)
```

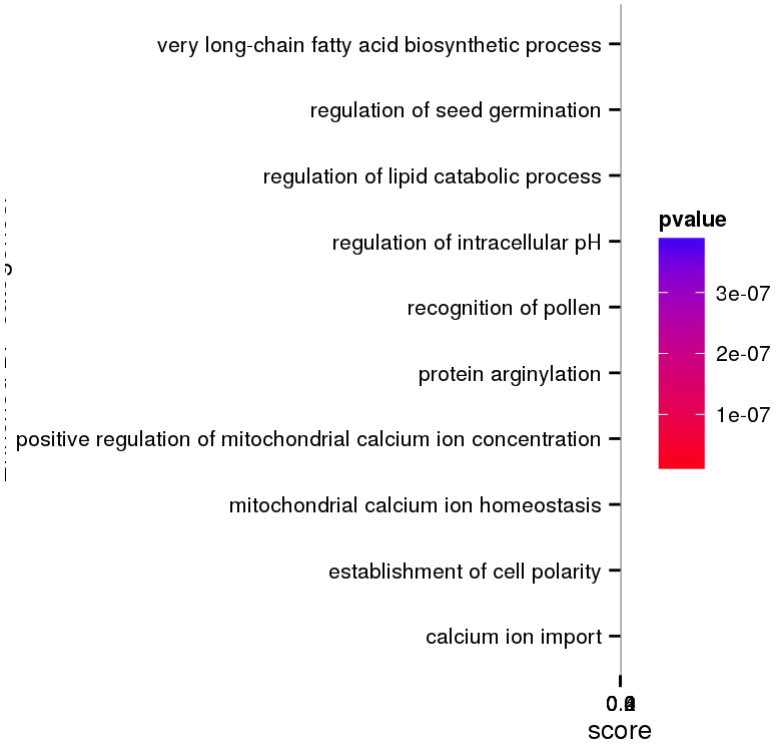
```
##        category over_represented_pvalue under_represented_pvalue
## 2415 GO:0008911            4.151763e-13                         1
## 714  GO:0004057            2.421000e-12                         1
```

```
## 2303 GO:0008559            2.259948e-10                        1
## 1243 GO:0005516            1.338025e-09                        1
## 5382 GO:0042626            2.460200e-08                        1
## 3138 GO:0010521            3.571023e-08                        1
##      numDEInCat numInCat       qvalue ont
## 2415         10       10 3.264946e-09  MF
## 714          12       16 4.759685e-09  MF
## 2303         23       86 2.538891e-07  MF
## 1243         40      232 1.169136e-06  MF
## 5382         42      281 1.138060e-05  MF
## 3138          8       11 1.560140e-05  MF
##                                                                 term
## 2415                              lactaldehyde dehydrogenase activity
## 714                                       arginyltransferase activity
## 2303                           xenobiotic-transporting ATPase activity
## 1243                                                 calmodulin binding
## 5382 ATPase activity, coupled to transmembrane movement of substances
## 3138                                      telomerase inhibitor activity
```

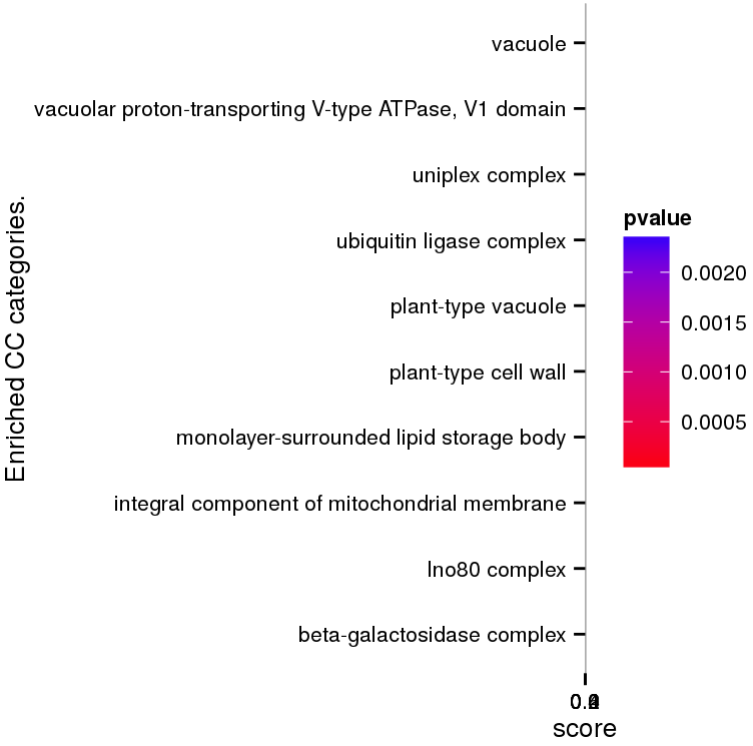
```
group_one_go$mfp_plot
```




```
group_one_go$bpp_plot
```




```
group_one_go$ccp_plot
```




```
## Print trees of the goseq() data
initial_trees = goseq_trees(group_one, group_one_go, goids_df=go_ids)
```

```
## Error in .local(.Object, ...): allGenes must be a factor with 2 levels
```

```
initial_trees$MF
```

```
## Error in eval(expr, envir, enclos): object 'initial_trees' not found
```

```
initial_trees$BP
```

```
## Error in eval(expr, envir, enclos): object 'initial_trees' not found
```

```
initial_trees$CC
```

```
## Error in eval(expr, envir, enclos): object 'initial_trees' not found
```

Vignette Info

Note the various macros within the `vignette` setion of the metadata block above. These are required in order to instruct R how to build the vignette. Note that you should change the `title` field and the `\VignetteIndexEntry` to match the title of your vignette.

Styles

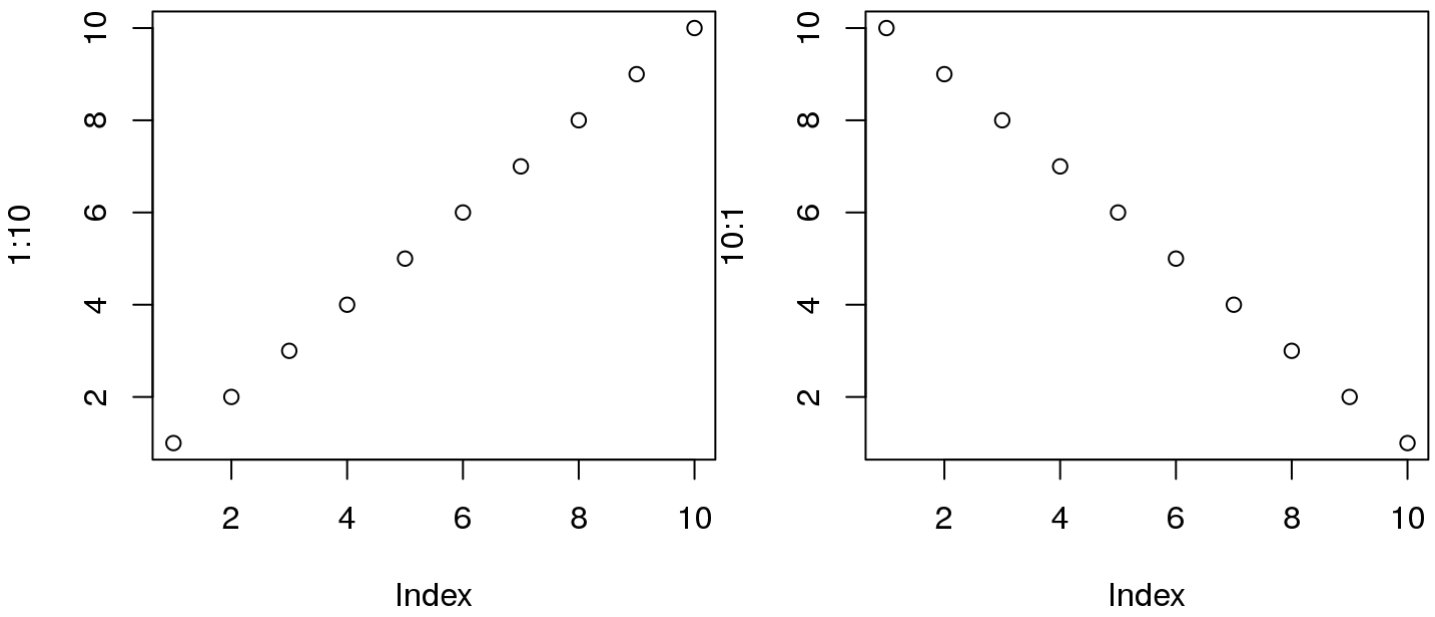
The `html_vignette` template includes a basic CSS theme. To override this theme you can specify your own CSS in the document metadata as follows:

```
output:
  rmarkdown::html_vignette:
    css: mystyles.css
```

Figures

The figure sizes have been customised so that you can easily put two images side-by-side.

```
plot(1:10)
plot(10:1)
```



You can enable figure captions by `fig_caption: yes` in YAML:

```
output:
  rmarkdown::html_vignette:
    fig_caption: yes
```

Then you can use the chunk option `fig.cap = "Your figure caption."` in **knitr**.

More Examples

You can write math expressions, e.g. $Y = X\beta + \epsilon$, footnotes[1], and tables, e.g. using `knitr::kable()`.

	mpg	cyl	disp	hp	drat	wt	qsec	vs	am	gear	carb
Mazda RX4	21.0	6	160.0	110	3.90	2.620	16.46	0	1	4	4
Mazda RX4 Wag	21.0	6	160.0	110	3.90	2.875	17.02	0	1	4	4
Datsun 710	22.8	4	108.0	93	3.85	2.320	18.61	1	1	4	1
Hornet 4 Drive	21.4	6	258.0	110	3.08	3.215	19.44	1	0	3	1
Hornet Sportabout	18.7	8	360.0	175	3.15	3.440	17.02	0	0	3	2
Valiant	18.1	6	225.0	105	2.76	3.460	20.22	1	0	3	1
Duster 360	14.3	8	360.0	245	3.21	3.570	15.84	0	0	3	4
Merc 240D	24.4	4	146.7	62	3.69	3.190	20.00	1	0	4	2
Merc 230	22.8	4	140.8	95	3.92	3.150	22.90	1	0	4	2
Merc 280	19.2	6	167.6	123	3.92	3.440	18.30	1	0	4	4

Also a quote using >:

[1] A footnote here.

"He who gives up [code] safety for [code] speed deserves neither." (via)