Homework 6

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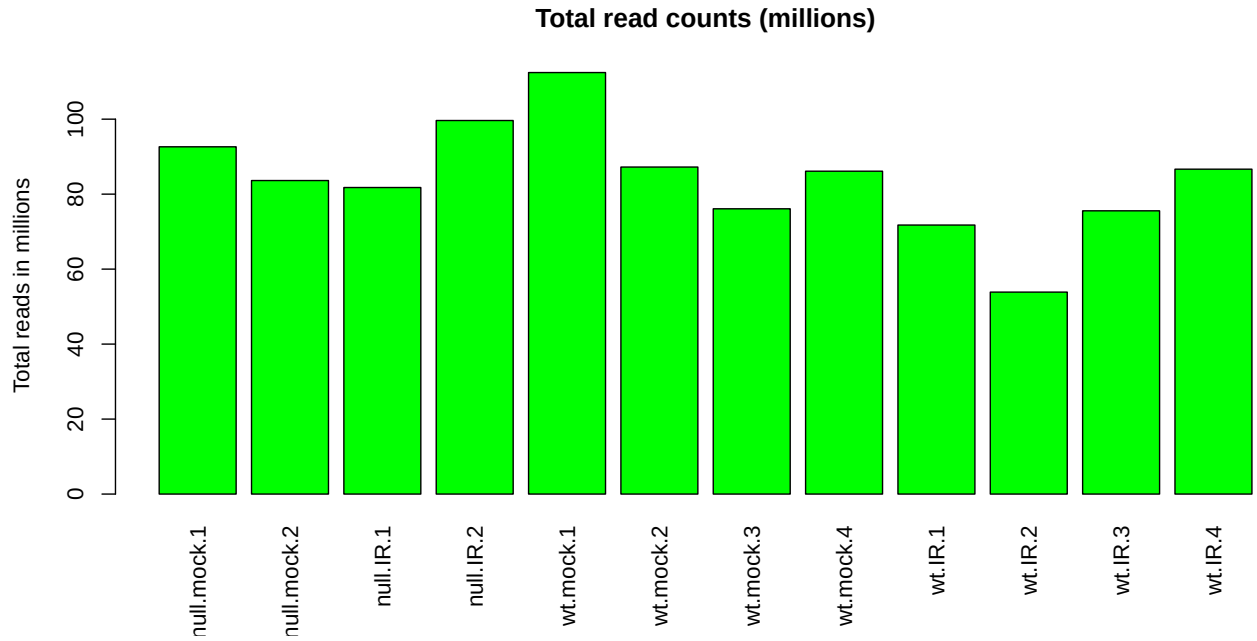
1. Reading data

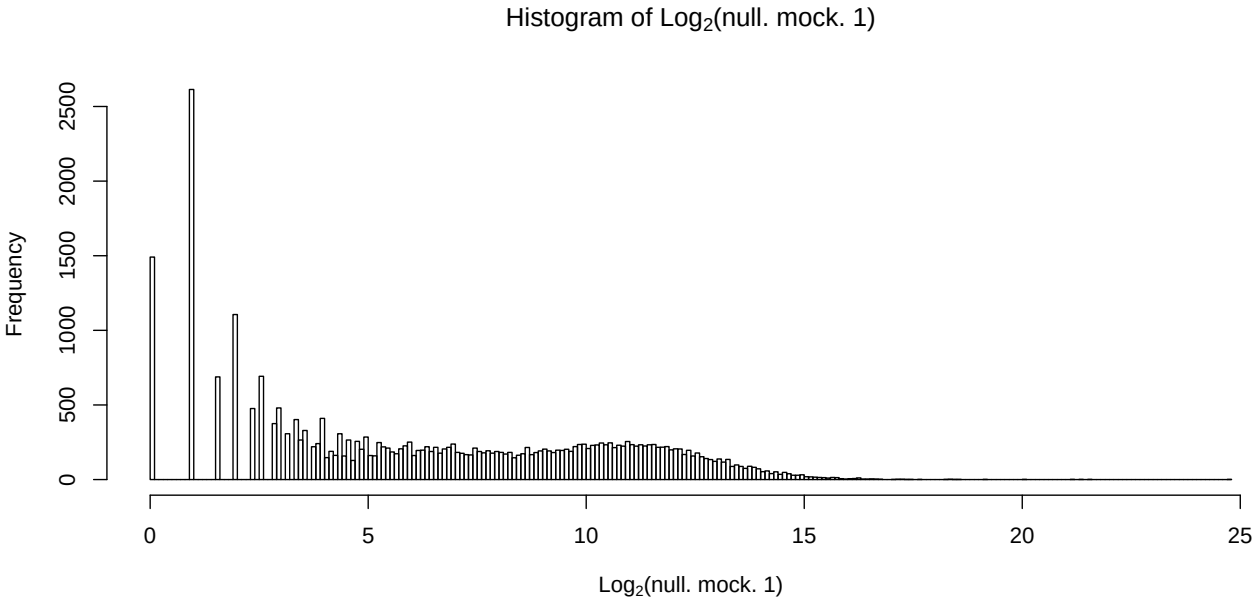
First we read in counts data inside the `readCounts.txt`.

2. Simple exploration of the raw data

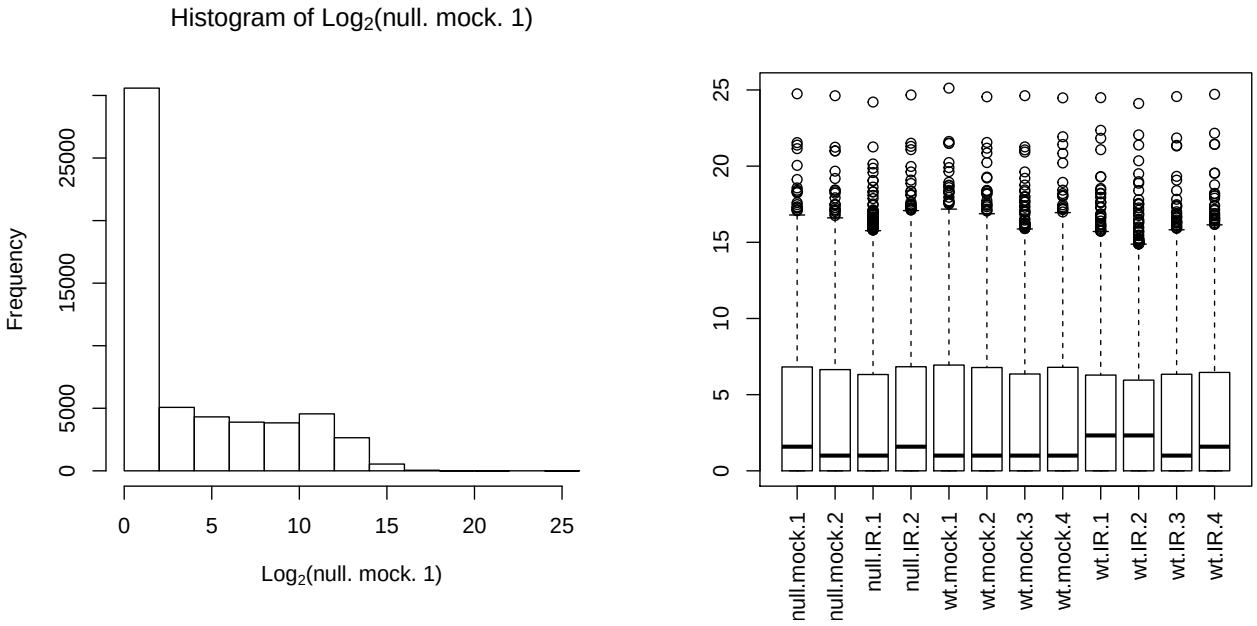
```
## [1] 55487    12
```

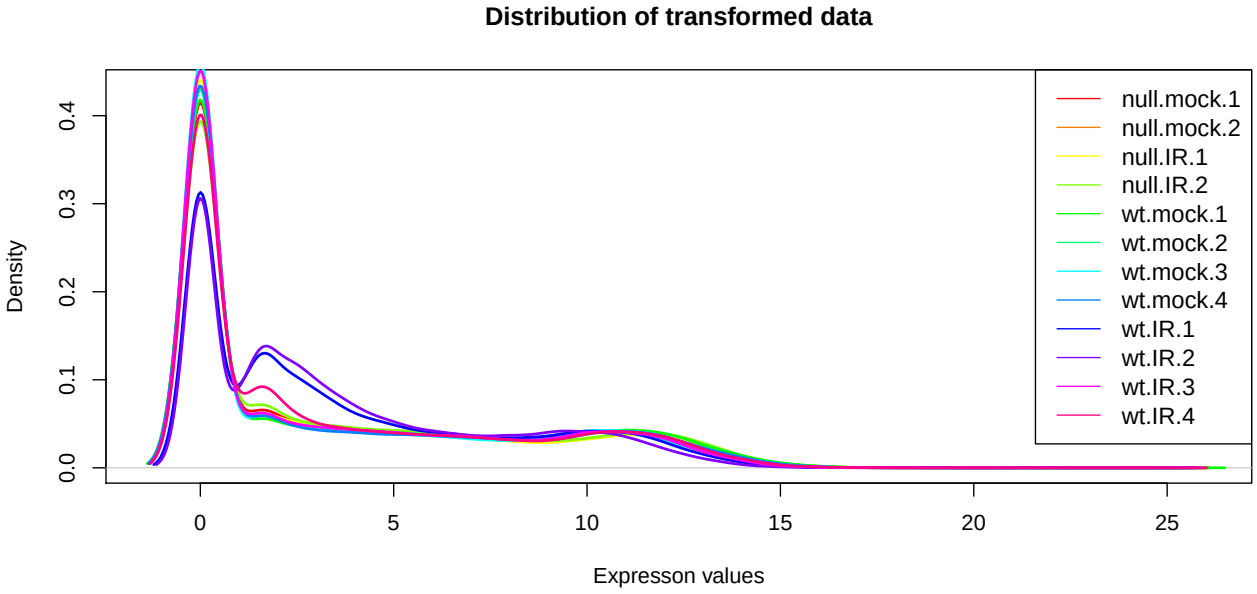
```
##   null.mock.1        null.mock.2         null.IR.1
##  Min.   :       0   Min.   :       0   Min.   :       0
##  1st Qu.:       0   1st Qu.:       0   1st Qu.:       0
##  Median :       2   Median :       1   Median :       1
##  Mean   :    1669   Mean   :    1507   Mean   :    1474
##  3rd Qu.:     112   3rd Qu.:      99   3rd Qu.:      79
##  Max.   :28184807   Max.   :25805512   Max.   :19350199
##    null.IR.2          wt.mock.1          wt.mock.2
##  Min.   :       0   Min.   :       0   Min.   :       0
##  1st Qu.:       0   1st Qu.:       0   1st Qu.:       0
##  Median :       2   Median :       1   Median :       1
##  Mean   :    1796   Mean   :    2026   Mean   :    1572
##  3rd Qu.:     113   3rd Qu.:     122   3rd Qu.:     109
##  Max.   :26735598   Max.   :36449709   Max.   :24558145
##    wt.mock.3          wt.mock.4             wt.IR.1
##  Min.   :       0   Min.   :       0   Min.   :       0
##  1st Qu.:       0   1st Qu.:       0   1st Qu.:       0
##  Median :       1   Median :       1   Median :       4
##  Mean   :    1371   Mean   :    1552   Mean   :    1293
##  3rd Qu.:      81   3rd Qu.:     110   3rd Qu.:      77
##  Max.   :25857899   Max.   :23413269   Max.   :23616031
##     wt.IR.2            wt.IR.3             wt.IR.4
##  Min.   :       0   Min.   :       0   Min.   :       0
##  1st Qu.:       0   1st Qu.:       0   1st Qu.:       0
##  Median :       4   Median :       1   Median :       2
##  Mean   :     971   Mean   :    1362   Mean   :    1562
##  3rd Qu.:      61   3rd Qu.:      80   3rd Qu.:      87
##  Max.   :18142468   Max.   :24872956   Max.   :27525525
```

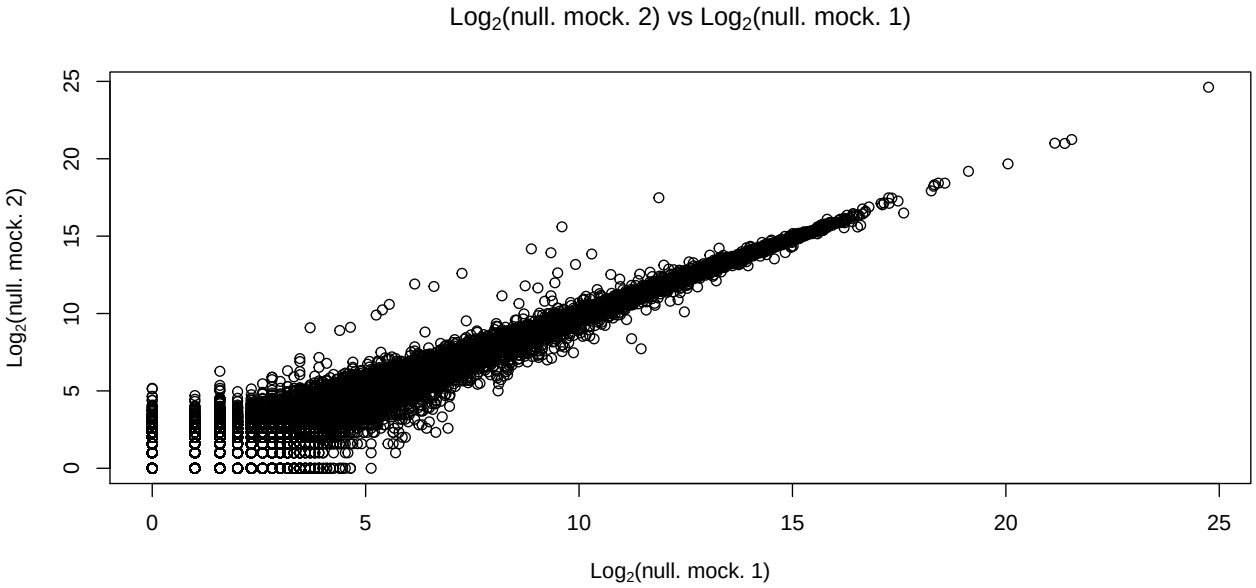

Total read counts (millions)
Total reads in millions
0
20
40
60
80
100
null.mock.1
null.mock.2
null.IR.1
null.IR.2
wt.mock.1
wt.mock.2
wt.mock.3
wt.mock.4
wt.IR.1
wt.IR.2
wt.IR.3
wt.IR.4


Histogram of Log2(null. mock. 1)
Frequency
0
500
1000
1500
2000
2500
0
5
10
15
20
25
Log2(null. mock. 1)

Simple log transformation on unnormalized data







3. Filtering, normalization, and transformation using DESeq2

Define sample groups

First we define a function for parsing samples into groups. Define sample groups based on column names

Now we need to add sample IDs, as this is very likely a paired situation. We add a new column this is according to this post

```
##                groups  p53 treatment
## null.mock.1 null.mock null      mock
## null.mock.2 null.mock null      mock
## null.IR.1     null.IR null        IR
## null.IR.2     null.IR null        IR
## wt.mock.1     wt.mock   wt      mock
## wt.mock.2     wt.mock   wt      mock
## wt.mock.3     wt.mock   wt      mock
## wt.mock.4     wt.mock   wt      mock
## wt.IR.1         wt.IR   wt        IR
## wt.IR.2         wt.IR   wt        IR
## wt.IR.3         wt.IR   wt        IR
## wt.IR.4         wt.IR   wt        IR
```

```
## 'data.frame':    12 obs. of  3 variables:
##  $ groups   : Factor w/ 4 levels "null.IR","null.mock",..: 2 2 1 1 4 4 4 4 3 3 ...
##  $ p53      : Factor w/ 2 levels "null","wt": 1 1 1 1 2 2 2 2 2 2 ...
##   ..- attr(*, "names")= chr  "null.mock.1" "null.mock.2" "null.IR.1" "null.IR.2" ...
##  $ treatment: Factor w/ 2 levels "IR","mock": 2 2 1 1 2 2 2 2 1 1 ...
##   ..- attr(*, "names")= chr  "null.mock.1" "null.mock.2" "null.IR.1" "null.IR.2" ...
```

Set up the DESeqDataSet Object and run the DESeq pipeline

```
## [1] 55487
```

Filtering

```
## [1] 36579
```

rlog transformation

```
##                       null.mock.1 null.mock.2   null.IR.1   null.IR.2
## ENSMUSG00000051951.5  0.09379245  0.09484254  0.09527086  0.09310978
## ENSMUSG00000103377.1 -0.57297871 -0.57239922 -0.57216237 -0.57335454
## ENSMUSG00000104017.1 -0.28881375 -0.28816033 -0.28789303 -0.28923708
##                        wt.mock.1   wt.mock.2   wt.mock.3   wt.mock.4
## ENSMUSG00000051951.5  0.0928600  0.09407265  0.09673701  0.09410612
## ENSMUSG00000103377.1 -0.5734919 -0.57282425 -0.57134954 -0.57280579
## ENSMUSG00000104017.1 -0.2893917 -0.28863966 -0.28697462 -0.28861885
##                           wt.IR.1     wt.IR.2     wt.IR.3     wt.IR.4
## ENSMUSG00000051951.5  0.26809062  0.58508811  0.09716842  0.09591506
## ENSMUSG00000103377.1 -0.19151931 -0.56245087 -0.57110974 -0.57180563
## ENSMUSG00000104017.1 -0.08419834 -0.03388829 -0.28670337 -0.28749015
```

VSD transformation

```
##                       null.mock.1 null.mock.2 null.IR.1 null.IR.2 wt.mock.1
## ENSMUSG00000051951.5    5.236118    5.236118  5.236118  5.236118  5.236118
## ENSMUSG00000103377.1    5.236118    5.236118  5.236118  5.236118  5.236118
## ENSMUSG00000104017.1    5.236118    5.236118  5.236118  5.236118  5.236118
##                       wt.mock.2 wt.mock.3 wt.mock.4  wt.IR.1  wt.IR.2
## ENSMUSG00000051951.5  5.236118  5.236118  5.236118 5.696882 6.102231
## ENSMUSG00000103377.1  5.236118  5.236118  5.236118 5.983363 5.236118
## ENSMUSG00000104017.1  5.236118  5.236118  5.236118 5.767419 5.853079
##                        wt.IR.3  wt.IR.4
## ENSMUSG00000051951.5 5.236118 5.236118
## ENSMUSG00000103377.1 5.236118 5.236118
## ENSMUSG00000104017.1 5.236118 5.236118
```

For the log2 approach, we need to first estimate size factors to account for sequencing depth, and then specify normalized=TRUE. Sequencing depth correction is done automatically for the rlog and the vst.

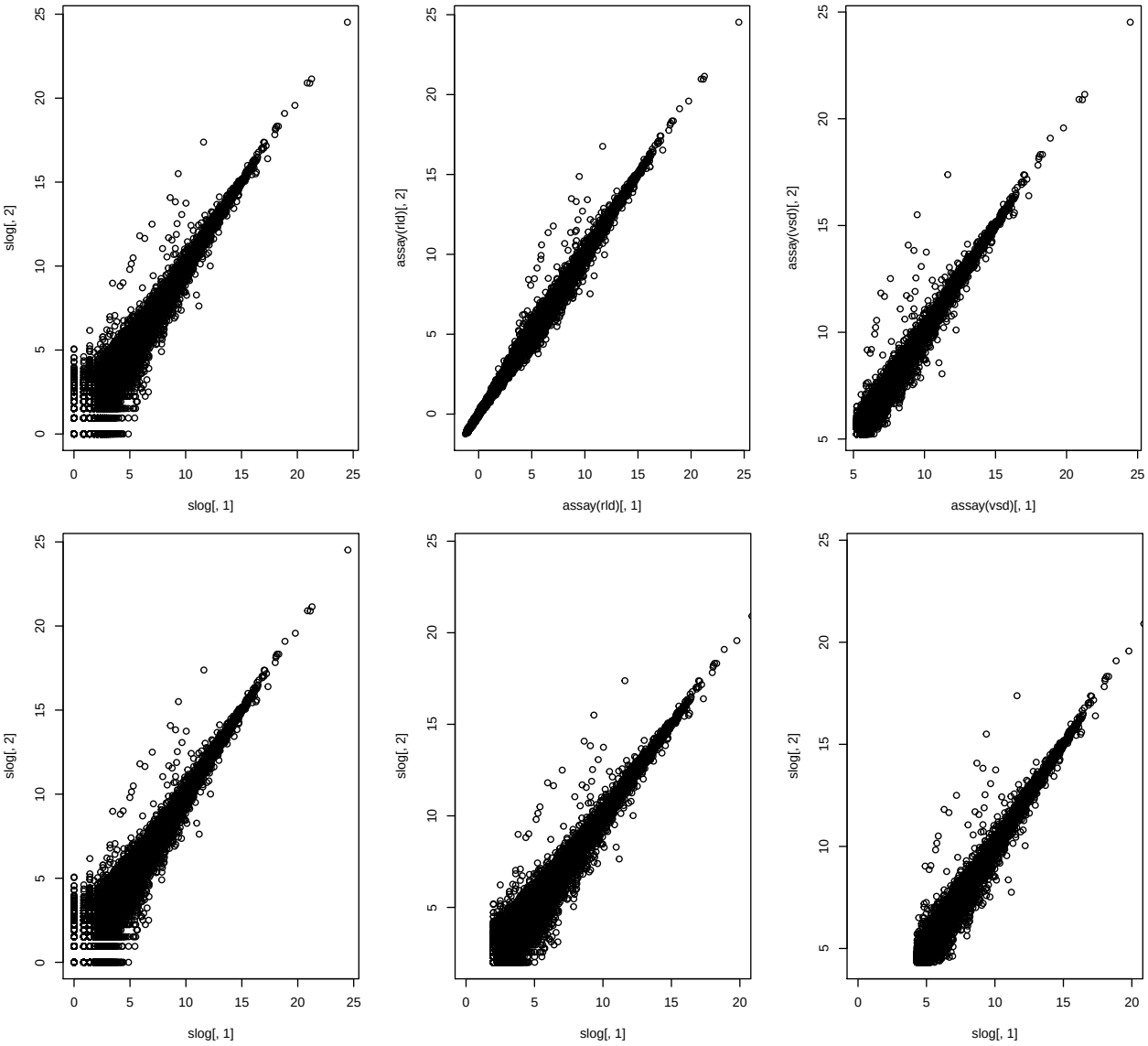
```
## null.mock.1 null.mock.2   null.IR.1   null.IR.2   wt.mock.1   wt.mock.2
##   1.2031419   1.0714551   1.0244459   1.3040657   1.3446410   1.1654621
##   wt.mock.3   wt.mock.4     wt.IR.1     wt.IR.2     wt.IR.3     wt.IR.4
##   0.8864428   1.1610935   0.7737430   0.5715530   0.8514457   0.9598173
```

Started log on scaled data

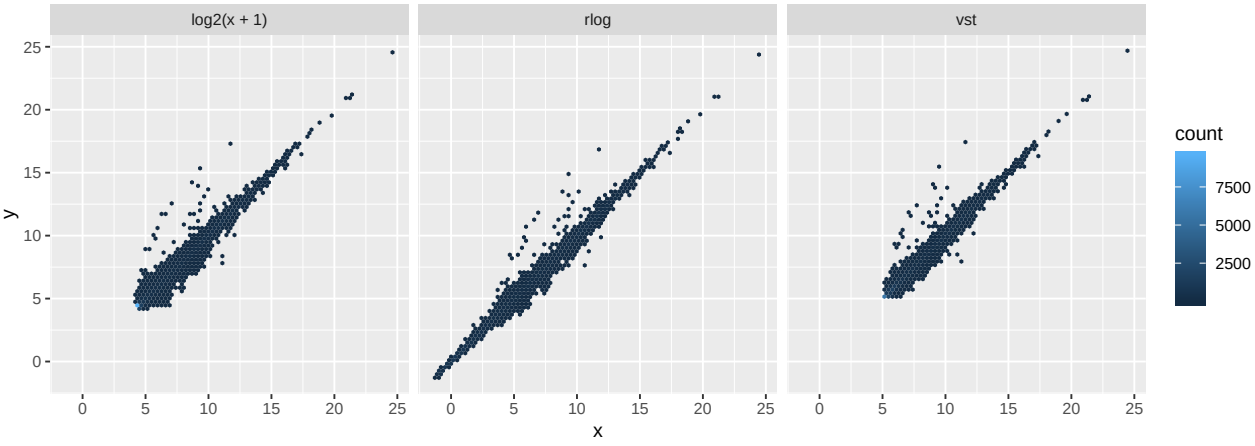
Usings the normalized=TRUE option in the counts( ) method of DESeq2, we adjust for different library sizes.

```
##                       null.mock.1 null.mock.2 null.IR.1 null.IR.2 wt.mock.1
## ENSMUSG00000051951.5           0           0         0         0         0
## ENSMUSG00000103377.1           0           0         0         0         0
## ENSMUSG00000104017.1           0           0         0         0         0
## ENSMUSG00000102331.1           0           0         0         0         0
## ENSMUSG00000102592.1           0           0         0         0         0
## ENSMUSG00000102343.1           0           0         0         0         0
##                       wt.mock.2 wt.mock.3 wt.mock.4  wt.IR.1  wt.IR.2
## ENSMUSG00000051951.5          0         0         0 2.286070 3.906597
## ENSMUSG00000103377.1          0         0         0 3.503266 0.000000
## ENSMUSG00000104017.1          0         0         0 2.625194 2.999725
## ENSMUSG00000102331.1          0         0         0 1.841908 2.999725
## ENSMUSG00000102592.1          0         0         0 4.045183 2.999725
## ENSMUSG00000102343.1          0         0         0 2.899580 0.000000
##                       wt.IR.3  wt.IR.4
```

```
## ENSMUSG00000051951.5       0 0.000000
## ENSMUSG00000103377.1       0 0.000000
## ENSMUSG00000104017.1       0 0.000000
## ENSMUSG00000102331.1       0 1.029887
## ENSMUSG00000102592.1       0 0.000000
## ENSMUSG00000102343.1       0 2.369455
```

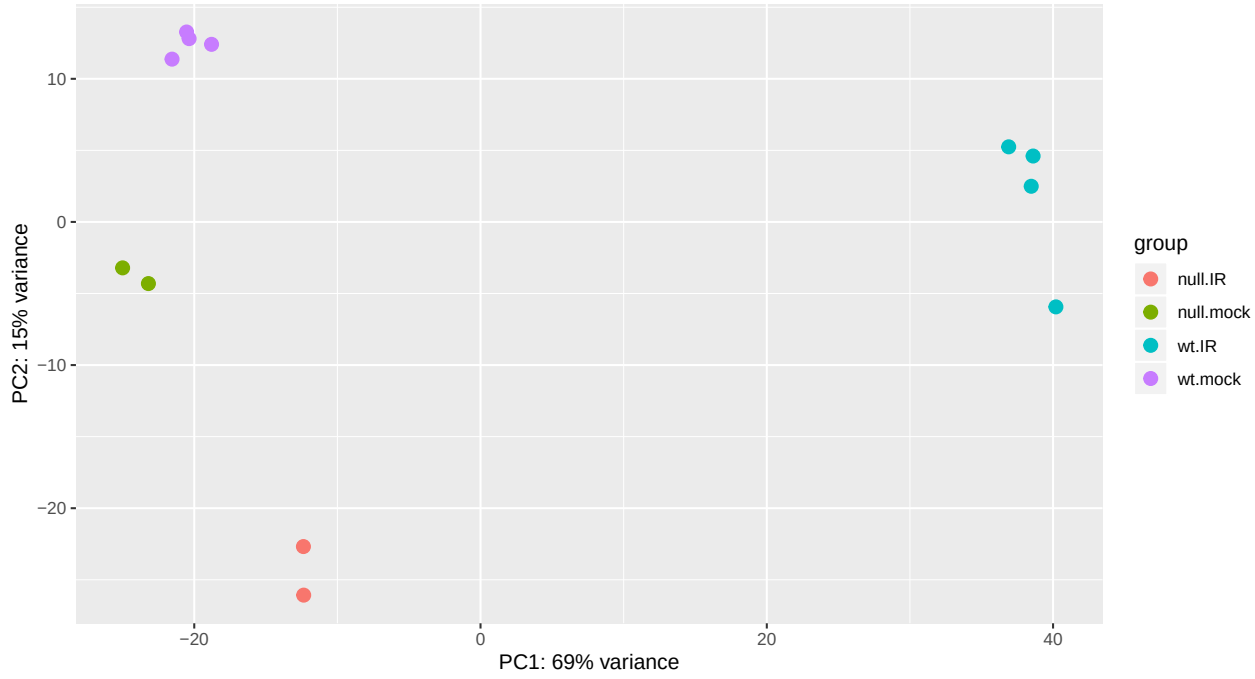


A more elegant plot using ggplot2 according to DESeq2 tutorial:

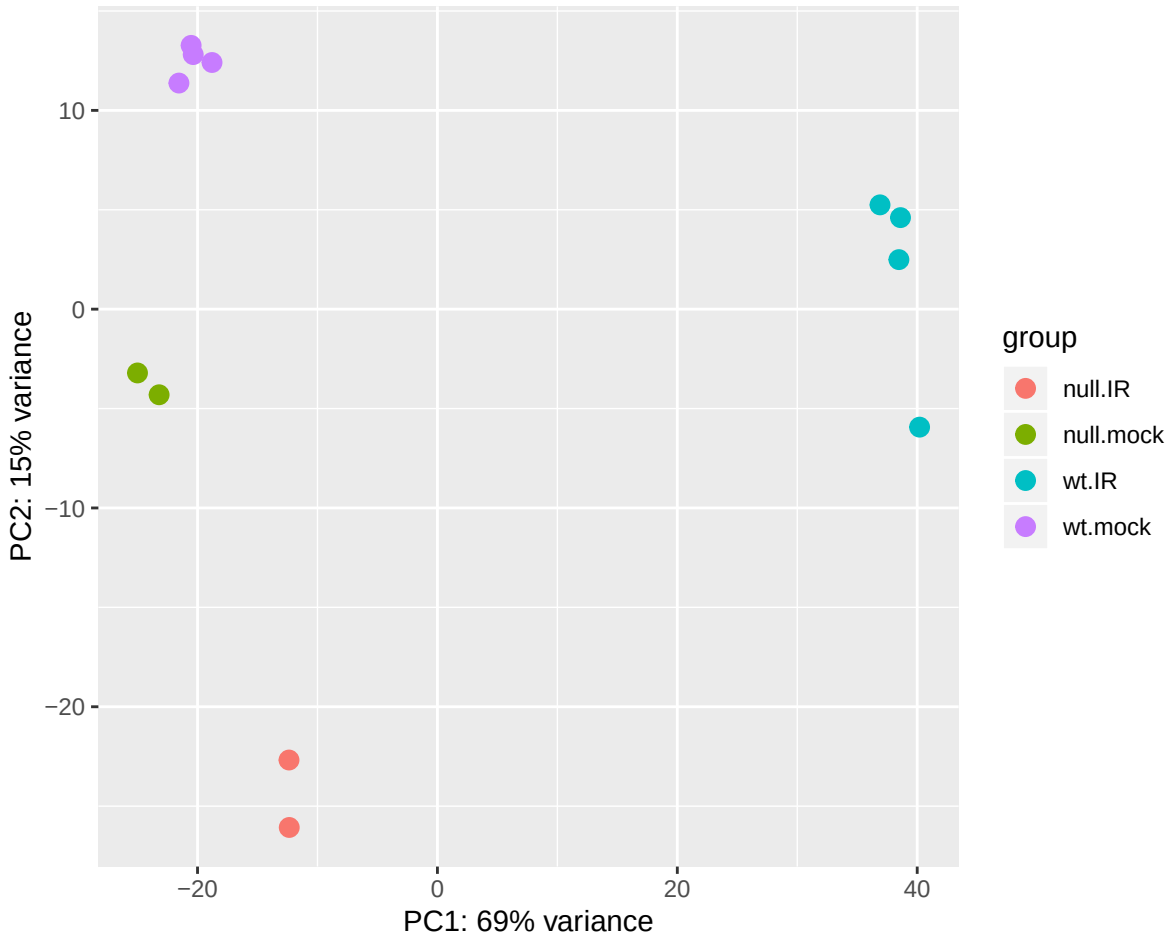


4. Exploratory Data Analysis

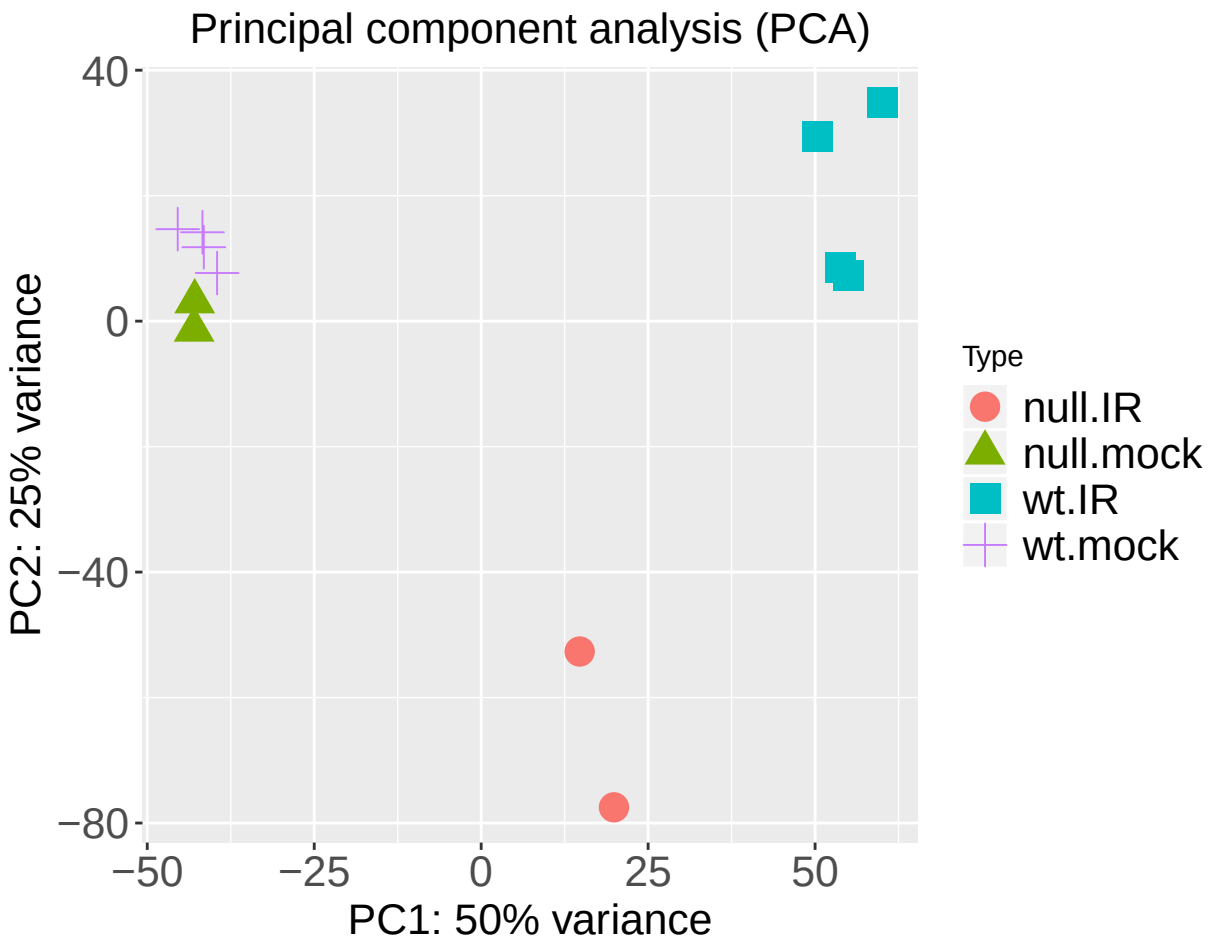
PCA plot



The figure looks odd. What we need is to make x and y aspect ratios independent of the data ranges.

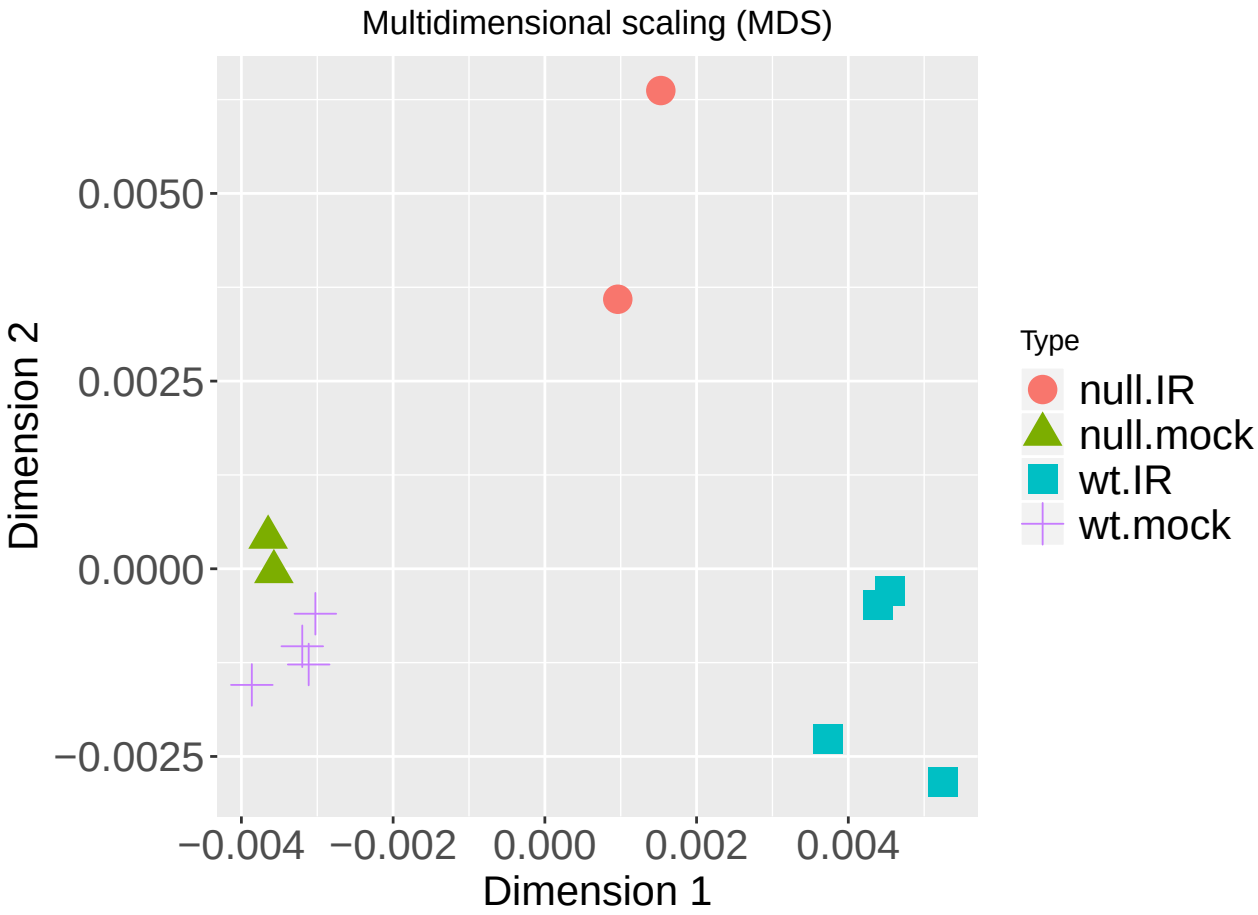


You can do the PCA plot yourself by building a plot using ggplot2, step by step

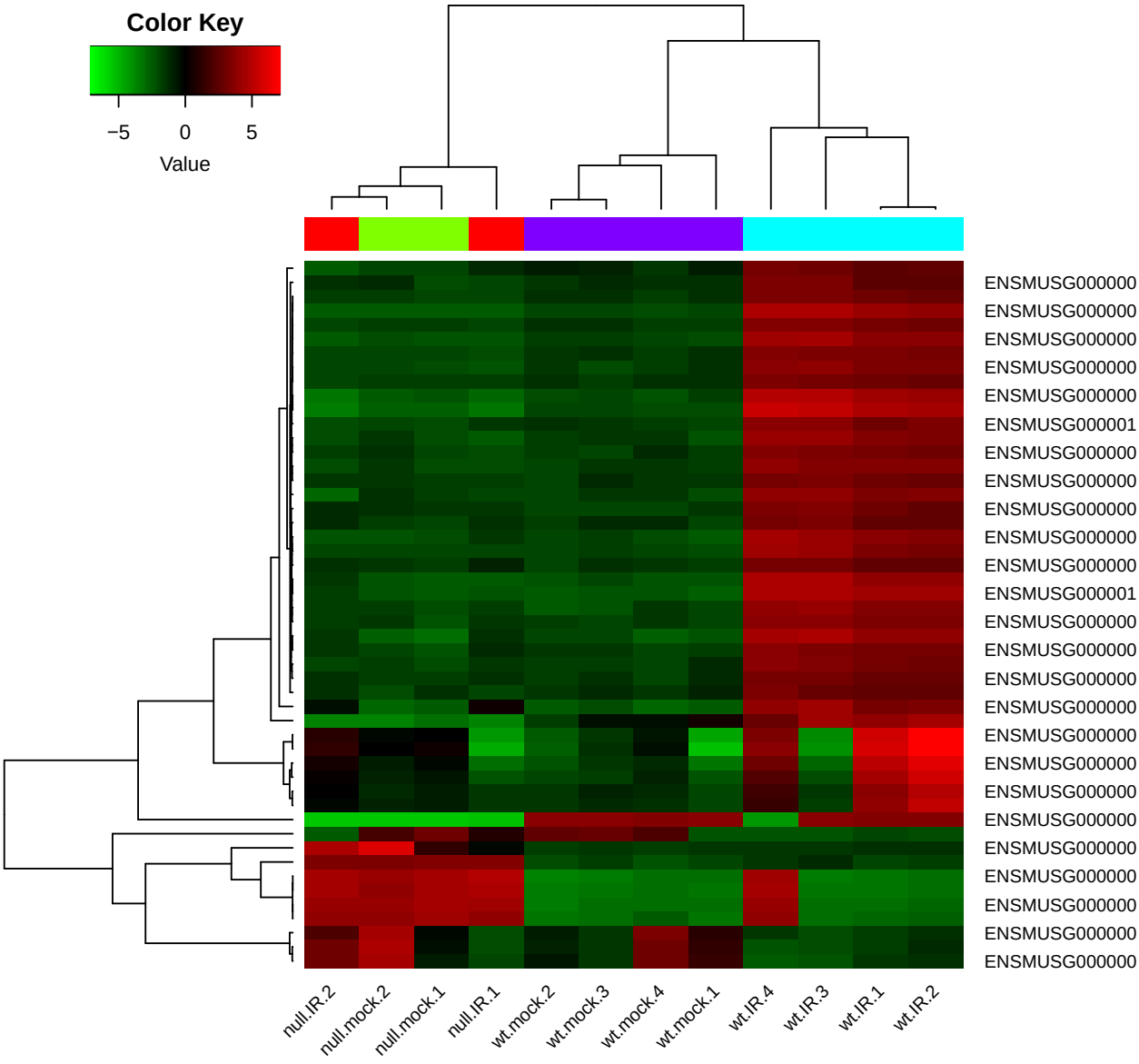


MDS plot

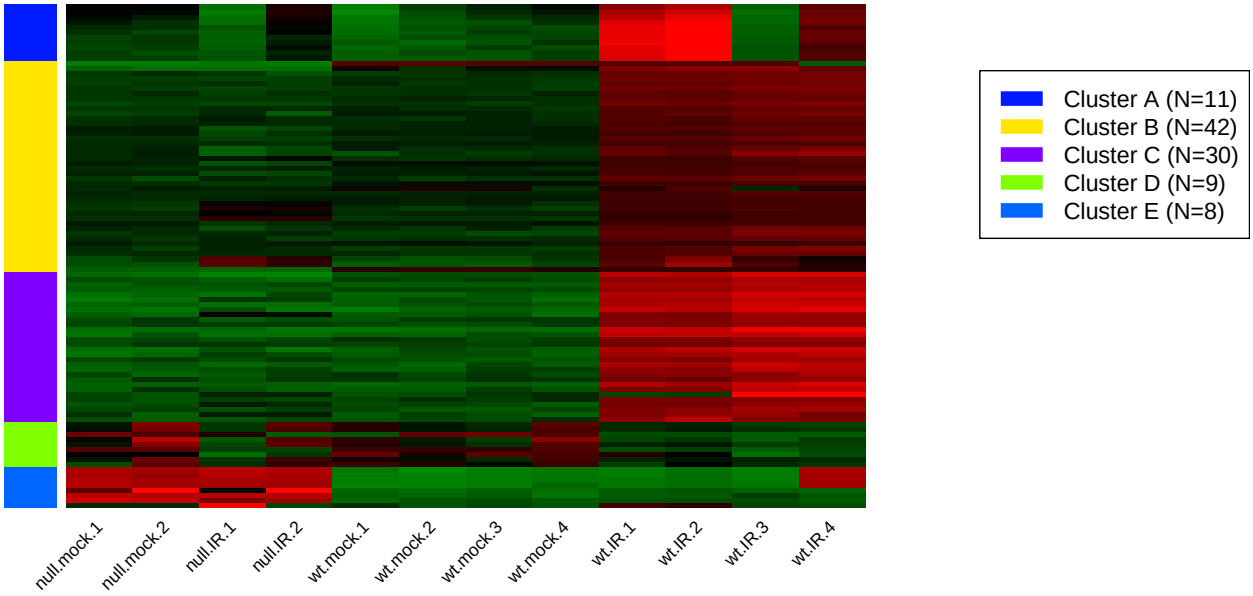
Similarily, we can produce MDS plots. As you can see from the code below, I recycled the ploting code and didn't even change some of the variable names.


Multidimensional scaling (MDS)
Dimension 2
0.0050
0.0025
0.0000
−0.0025
−0.004
−0.002
0.000
0.002
0.004
Dimension 1
Type
null.IR
null.mock
wt.IR
wt.mock

Hierarchical clustering with heatmap



K-means clustering of genes



5. Differential expression analysis using DESeq2

```
## log2 fold change (MLE): treatment mock vs IR
## Wald test p-value: treatment mock vs IR
## DataFrame with 36579 rows and 6 columns
##                              baseMean     log2FoldChange
##                             <numeric>          <numeric>
## ENSMUSG00000051951.5  1.48951739302888  -3.45769667767622
## ENSMUSG00000103377.1 0.861612478553997  -2.78547912179598
## ENSMUSG00000104017.1  1.01401259606256  -2.95855679102816
## ENSMUSG00000102331.1 0.885431555118192  -2.80787322518581
## ENSMUSG00000102592.1  1.87562507461656  -3.77830056153258
## ...                                ...                ...
## ENSMUSG00000064368.1  48761.3379686471  -0.36069784835848
## ENSMUSG00000064369.1  3148.20650066707  0.248995813844674
## ENSMUSG00000064370.1  229153.247939432 -0.463149607155602
## ENSMUSG00000064371.1  54.7512721213404  -0.61944345152823
## ENSMUSG00000064372.1  942.383128434213 -0.870667263888078
##                                lfcSE               stat
##                            <numeric>          <numeric>
## ENSMUSG00000051951.5  3.04592047170739  -1.13518941475777
## ENSMUSG00000103377.1  3.06610664118698 -0.908474312138615
## ENSMUSG00000104017.1   3.0612051051142 -0.966468005062925
## ENSMUSG00000102331.1  2.67830291137901   -1.0483777668524
## ENSMUSG00000102592.1  2.93580169068832  -1.28697403966912
## ...                                ...                ...
## ENSMUSG00000064368.1 0.238976299912887  -1.50934569030471
## ENSMUSG00000064369.1 0.159271605309944   1.56334089406663
## ENSMUSG00000064370.1 0.213827759595239  -2.16599382620999
## ENSMUSG00000064371.1   0.3060705555512   -2.0238583564913
## ENSMUSG00000064372.1 0.202240636016501  -4.30510544783413
##                                 pvalue                 padj
```

```
##                                 <numeric>            <numeric>
## ENSMUSG00000051951.5    0.256295963671824    0.376836207666776
## ENSMUSG00000103377.1    0.363627679571275                   NA
## ENSMUSG00000104017.1    0.333810049356437                   NA
## ENSMUSG00000102331.1    0.294464592857858                   NA
## ENSMUSG00000102592.1    0.198103336743242    0.312377384457178
## ...                                   ...                  ...
## ENSMUSG00000064368.1    0.131210464193762    0.228775700116307
## ENSMUSG00000064369.1    0.117972434432238    0.211583081591477
## ENSMUSG00000064370.1   0.0303116587152136    0.072138804635945
## ENSMUSG00000064371.1   0.0429847277621139   0.0962055462138653
## ENSMUSG00000064372.1 1.66906214208969e-05 9.45648783641496e-05
```

Applying cutoff

DESeq2 uses the Benjamini-Hochberg (BH) adjustment (Benjamini and Hochberg 1995) as implemented in the base R p.adjust function

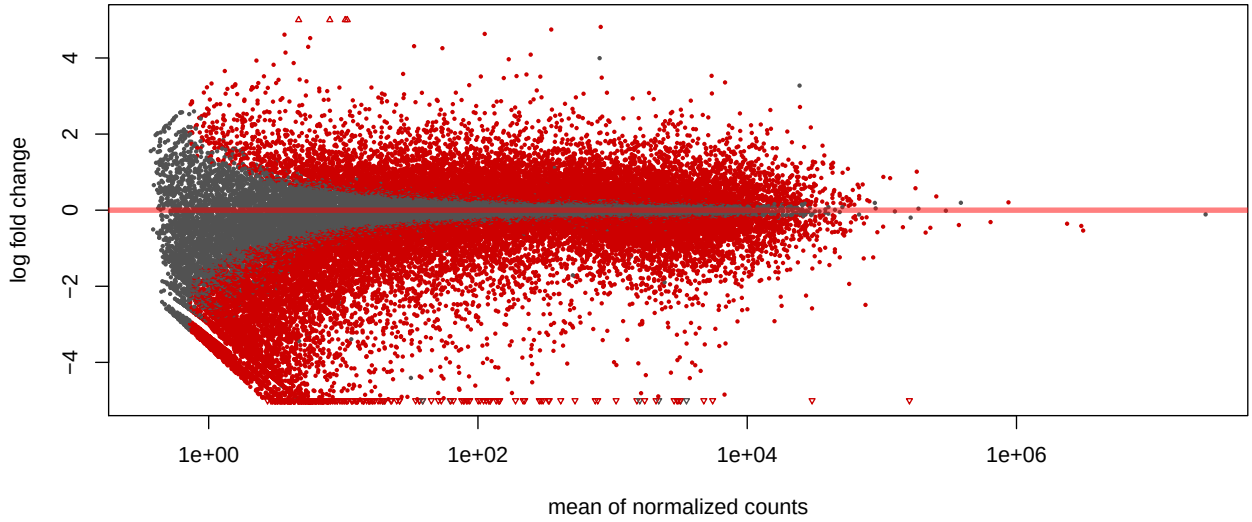
```
##
## out of 36579 with nonzero total read count
## adjusted p-value < 0.5
## LFC > 0.01 (up)    : 8857, 24%
## LFC < -0.01 (down) : 15557, 43%
## outliers [1]       : 22, 0.06%
## low counts [2]     : 1334, 3.6%
## (mean count < 1)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

##Sort genes by fold change

```
## log2 fold change (MLE): treatment mock vs IR
## Wald test p-value: treatment mock vs IR
## DataFrame with 6 rows and 6 columns
##                              baseMean    log2FoldChange             lfcSE
##                             <numeric>         <numeric>         <numeric>
## ENSMUSG00000026831.16 294.434890082783 -10.8986209218927  2.39452946470452
## ENSMUSG00000000308.14 115.338446153961  -9.2173382850945  1.54573375190591
## ENSMUSG00000087132.8  50.8350280621933 -8.25552375037134  1.93318328778446
## ENSMUSG00000114192.1  26.2235736013177 -8.17831689044707  1.08940650090745
## ENSMUSG00000034818.16 525.897102370369 -7.80808045725016 0.917981250446441
## ENSMUSG00000069049.11 2201.07598176852 -7.36932902833692   2.5869262636059
##                                  stat               pvalue
##                             <numeric>            <numeric>
## ENSMUSG00000026831.16 -4.54729043112287 5.43409860442426e-06
## ENSMUSG00000000308.14 -5.95661333896718 2.57518541118292e-09
## ENSMUSG00000087132.8  -4.26525710338681 1.99672135724724e-05
## ENSMUSG00000114192.1   -7.4979513006789 6.48229732567845e-14
## ENSMUSG00000034818.16 -8.49481452203706 1.98249242800415e-17
## ENSMUSG00000069049.11 -2.84481592377464                   NA
##                                    padj
##                               <numeric>
## ENSMUSG00000026831.16 3.92086476779822e-05
## ENSMUSG00000000308.14 3.15059936568587e-08
## ENSMUSG00000087132.8  0.000129641504822709
## ENSMUSG00000114192.1   1.3680404955205e-12
```

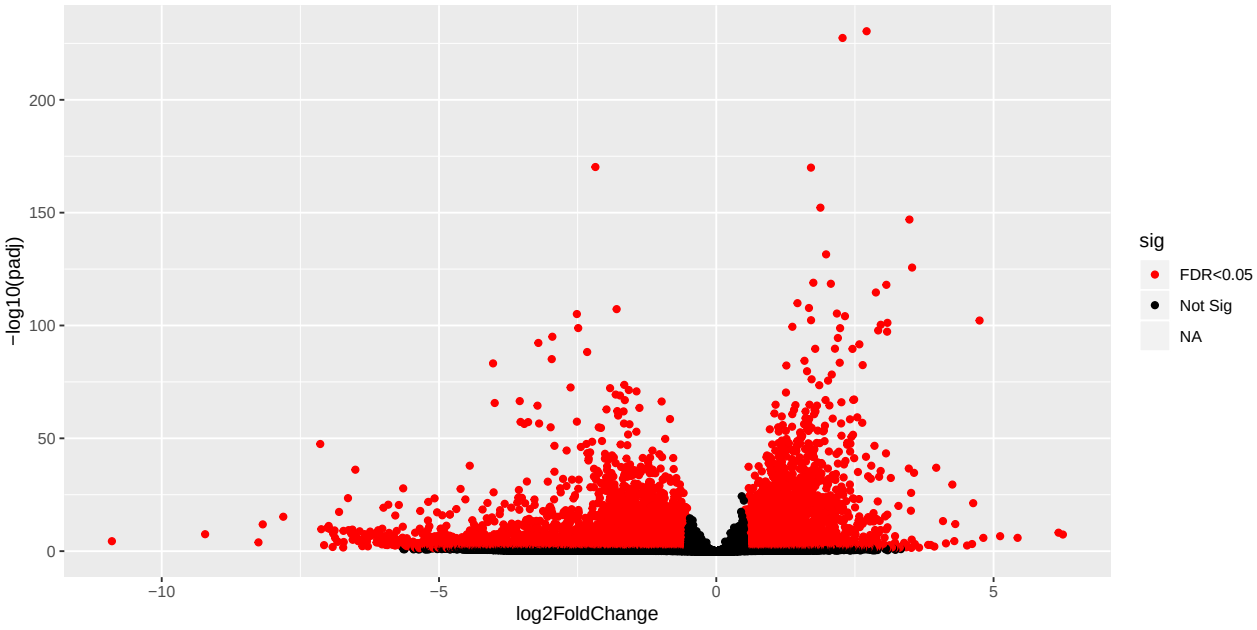
```
## ENSMUSG00000034818.16 5.67718136516993e-16
## ENSMUSG00000069049.11                   NA
```

MA plot

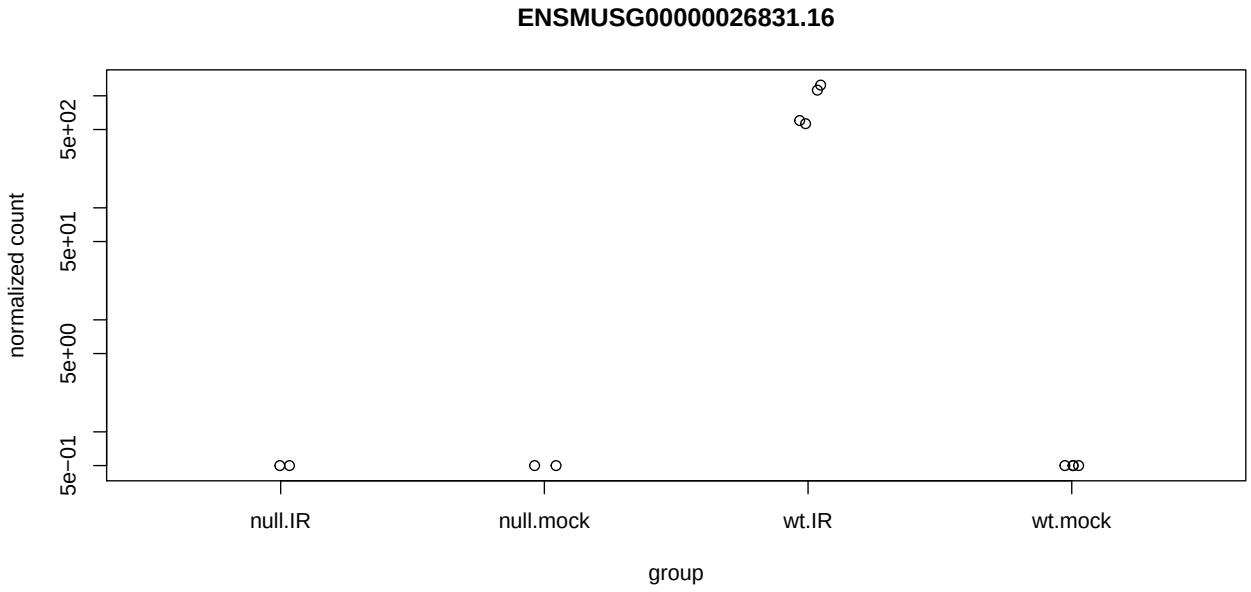


DESeq2 also provides shrinked log fold changes:

Basic volcano plot



Ploting counts of selected genes



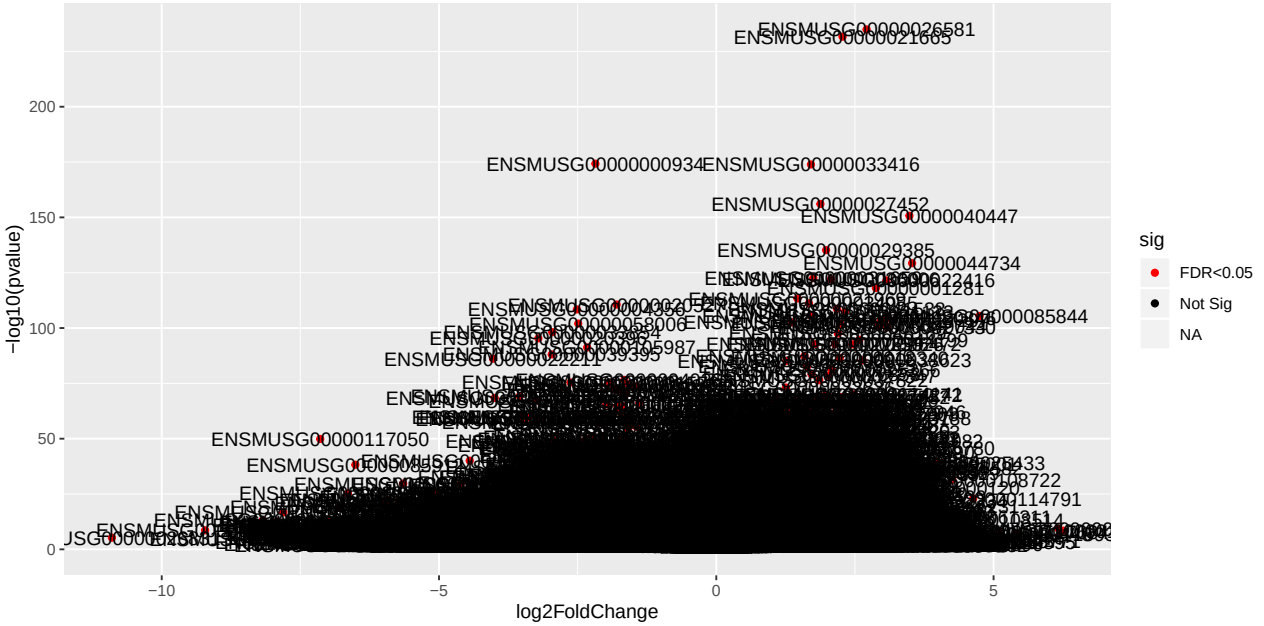
6. Annotating genes using Bioconductor

First we need to install Bioconductor base and some Bioconductor packages.

That file contains all genes passed filter. Let's output only the significant genes. We also sorted the genes by fold change.

```
##                                 symbol   baseMean log2FoldChange
## ENSMUSG00000107262.1 ENSMUSG00000107262   7.938182       6.250145
## ENSMUSG00000086137.1 ENSMUSG00000086137  10.699320       6.171791
## ENSMUSG00000116939.1 ENSMUSG00000116939   4.660927       5.433744
## ENSMUSG00000117684.1 ENSMUSG00000117684  10.347561       5.118549
## ENSMUSG00000080440.1 ENSMUSG00000080440 816.154550       4.816159
## ENSMUSG00000085844.1 ENSMUSG00000085844 350.345774       4.747714
##                          lfcSE      stat        pvalue          padj
## ENSMUSG00000107262.1 1.0575439  5.900601  3.621792e-09  4.337653e-08
## ENSMUSG00000086137.1 0.9900713  6.223583  4.859269e-10  6.567845e-09
## ENSMUSG00000116939.1 1.0280289  5.275867  1.321299e-07  1.254112e-06
## ENSMUSG00000117684.1 0.9128756  5.596106  2.192192e-08  2.351982e-07
## ENSMUSG00000080440.1 0.9125607  5.266673  1.389185e-07  1.315003e-06
## ENSMUSG00000085844.1 0.2164780 21.885434 3.575948e-106 6.297780e-103
##                            entrez
## ENSMUSG00000107262.1       Gm18753
## ENSMUSG00000086137.1       Gm16248
## ENSMUSG00000116939.1       Gm49619
## ENSMUSG00000117684.1 4930449E18Rik
## ENSMUSG00000080440.1       Gm25848
## ENSMUSG00000085844.1       Gm11690
```

Enhanced volcano plot with gene symbols



Further improved volcano plot with ggrepel