Exploring RNA-seq data from Gierlinski, Schurch and Barton

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## Parameters for the execution of this tutorial

## Introduction

The goal of this tutorial is to get familiar with RNA-seq data:

* load a table of read counts per gene;
* do some exploratory statistics about these counts per gene (distributions, histograms, ...)

### Data source

The 48-replicate yeast study was obtained from Goeff Barton's group publications (Gierliński et al. 2015, Schurch et al. (2015)).

### Methods

Fang and coworkers (2012) compared different statistical models and tests (Poisson, Fisher, negative binomial, ...) for the detection of differentially expressed genes.

#### Full original datasets (beware, there are 672 fastq files !)

Data shared at ENA: <https://figshare.com/articles/Metadata_for_a_highly_replicated_two_condition_yeast_RNAseq_experiment_/1416210>

Fastq files: <http://www.ebi.ac.uk/ena/data/view/ERP004763>

#### Read count tables

* Wild-type yeast strains (WT): <https://dx.doi.org/10.6084/m9.figshare.1425503>
* Snf2 mutant: <https://dx.doi.org/10.6084/m9.figshare.1425502>

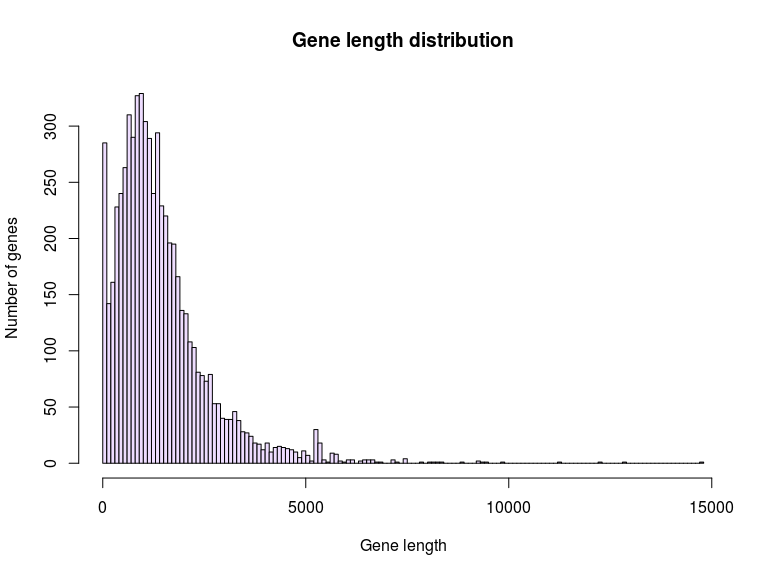
## Data loading

We will now load the two read count tables (WT and Snf2 mutants respectively).

## Gene annotation exploration

**Exercise for Mustafa:**

1. Count the number of genes in the gene.descriptions data frame.
2. Add a column to this data frame with the length of each gene.
3. Draw an histogram with the distribution of gene lengths.
4. Draw a box plot with the distribution of gene lengths.
5. Compute the min, max, mean, median of all the gene lengths.
6. Based on the column chrom.id, count the number of reads per chromosome (use the function table()), and represent it in a barplot.
7. Using the R function grep(), select all the genes whose name (namecolumn of the gene.description data frame) contains the string "SNF".



Length distribution for all the yeast genes

## Data exploration

We will first check the dimension of the two tables.

[1] 7125 48

[1] 7125 48

The tables *counts.wt* and *counts.snf2* contain the read counts per gene for the Wild-Type and the Snf2 strains, respectively. Each table contains one row per gene and one column per sample.

|  |  |  |
| --- | --- | --- |
| Genotype | Rows | Columns |
| WT | 7125 | 48 |
| Snf2 | 7125 | 48 |
| All | 7125 | 96 |
|  |  |

## Summary of counts per sample (column)

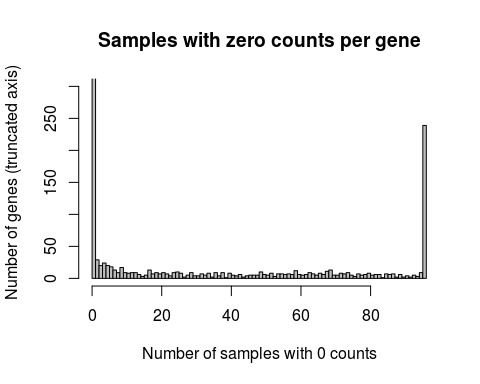
We could use the function *summary()* to compute descriptie statistics for each column of the count table.

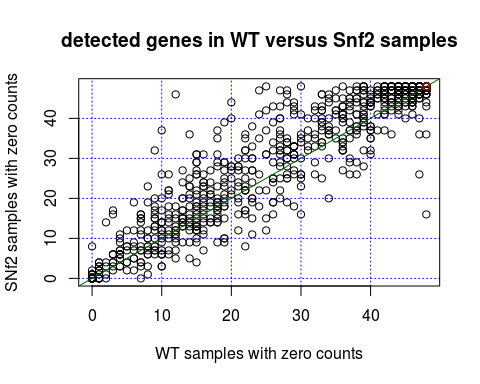
We can immediately draw some observations from these summaries.

* **min**: in each column, the mean is 0. This corresponds to genes that were undetected: apparently these genes are either not transcribed, or they are transcribed at a weak level so that they were not sequenced in the library.
* **Mean**: in this experiment the mean counts per gene are of the order of 1000 counts per gene for each biological sample.
* \*\*max\* The max count is immensely larger than the mean counts, and this is true for each column. This observation is very general with RNA-seq data: in all the cases I have studied so far, we detect a very few genes having a huge number of counts.
* **median** is the count value that splits the sorted values in two. Half of the values of a column are smaller than or equal to the median.

## Number of undetected genes per sample

We would like to count the number of undetected genes per sample, i.e. genes having zero counts.





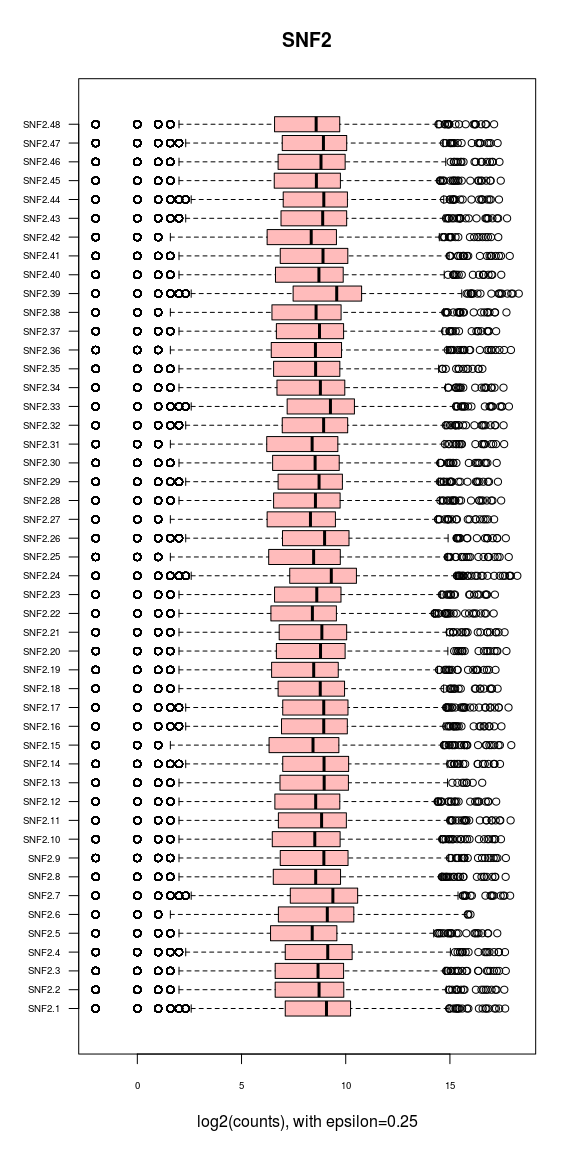
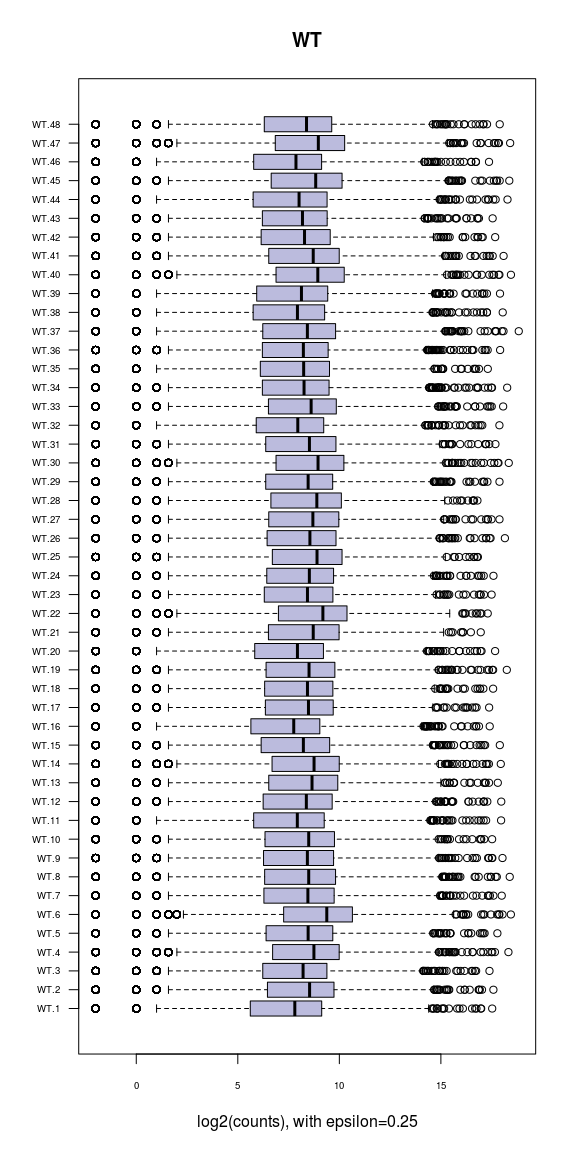
## Compute the log-transformed counts

RNA-seq count data are characterized by a very wide range of values, with small count numbers of the majority of the genes, and a small number of genes having hundreds fo thousands of counts. For many purposes (visualisation, summarization, ...), it is more relevant to work with log2-transformed counts.

However, we first must treate a problem: since many genes have 0 counts, the logarithmic transformation would give -Infinite, which creates problems for both computation and display. To circumvent this, we arbitrarily convert the zero counts in a small epsilon value (smaller than 1, so we can distinguish the genes with 1 counts from genes with 0 counts). we will choose an epsilon of 0.01.

## Box plots

The boxplots below summarizes the distribution of log2-transformed counts per genes for each sample.



## Normalization of RNA-seq counts

The total number of counts (``libsum'') can show wide variations from sample to sample, resulting from various sources (biological sample, sequencing, mapping, ...). Before comparing the counts per gene between different samples, it is important to perform a between-sample normalization.

Several methods have been proposed for this.

### Counts per million (CPM)

Counts are converted to counts per million by a simple scaling rule.

We dispose of a raw count table containing 7125 rows (one row per gene) and 96 columns (one column per sample).

Let us define as the count of reads for gene ( row) in sample ( column).

We can compute the sum of counts for each sample (column), which is also called the ***libsum***.

where is the total number of genes (rows in the table).

The basic way to compute counts per million reads is to divide each count by the libsum of the corresponding sample (column), and multiplying by 1 million for scaling purposes.

### Median-based standardization

A well-known problem with this simple approach is that the libsum can be strongly influenced by outliers, i.e. a handful of genes which are represented by hundreds of millions of reads.

To circumvent this, one possibility is to use a more robust scaling factor for each sample, for example the median count, or the percentile.

### When should we use / not use normalized counts?

Normalized log2-transformed counts are very convenient for display purposes (e.g. to compare the read density between samples or between conditions).

**However**, they cannot be used for all purposes. Indeed, the normalization procedure converts integer counts into fractional numbers, which do not comply with the probabilistic models underlying manysome of the statistical tests that are performed on these datasets. In particular, the most popular packages for differential analysis with RNA-seq expect to take as input a table with raw counts.

The log2 transformation completely modifies the nature of the data, and completely change the scale of the counts. Consequently, log2-transformed data should not be used either as input for the classical differential analysis packages.

Log2-transformed standardized counts may also be interesting for some clustering or classification purposes, because they will strongly reduce the dynamic range of the data, and the *log2 transformation has a normalizing effect* (this is a general effect) that may contribute to increase the suitedness of the data for some analysis methods (e.g. PCA, clustering, discriminant analysis, ...). We will investigate this very soon.

# The aim of Normalization

Is to remove systematic technical effects that occur in the data to ensure that technical bias has minimal impact on the results, and to equates the read cout distribution across samples.

## The Trimmed mean of M-Values (TMM) normalization method

Robinson and Oshlack (Robinson and Oshlack 2010) modeled the relationship between RNA quantities produced by a sample and RNA-seq counts.

Counts:

* = observed read counts for gene in sample .
* = total number of reads (all genes together) for sample

Actual RNA quantities per sample:

* = expected read counts for gene in sample .
* = true (but unknown) number of transcripts for gene in sample .
* = length of the gene
* = total RNA (all genes together) for sample .

Quote from Robinson and Oshlack (2010): "The total RNA dataset production, cannot be estimated directly, since we don't know the expression levels and true lengths of every gene."

Actually we know (approximately) the length o each gene as far as it has been correctly annotated in the genome, but we have no idea about the actual number of RNA molecules produced by each gene () in a given sample ().

"One simple yet robust way to estimate the ratio of RNA production uses a weighted trimmed mean of the log expression ratios (trimmed mean of M values (TMM))."

Computation of the and values

The value is the log2-ratio of relative counts for gene between two samples and .

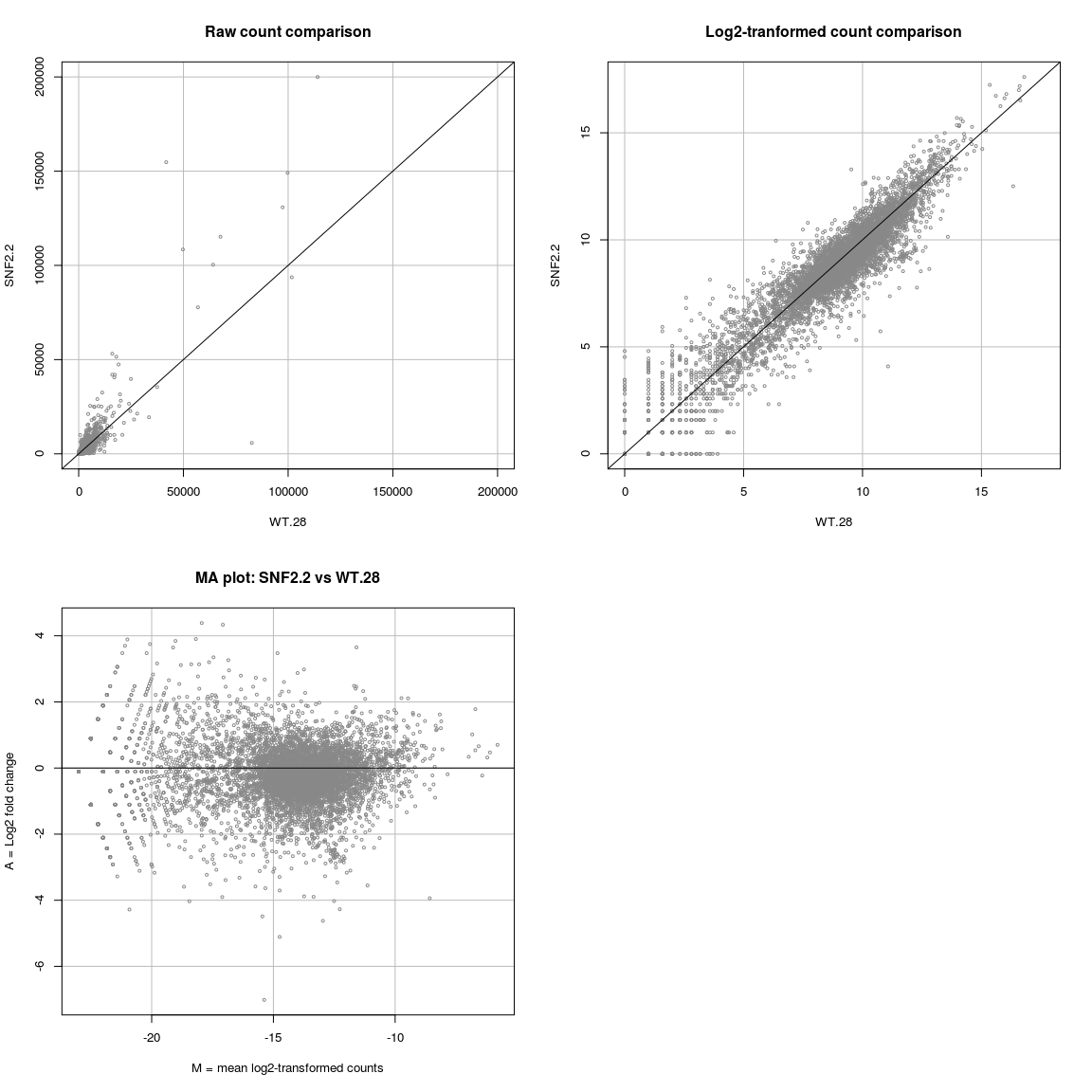
is the index of the "reference sample". Robinson and Oshlack indicate that for multi-sample analyses, one sample should be selected as reference, and the TMM should be computed for all the other samples, based on this reference. However the authors do not give clues about the way to choose "the" reference sample. In our case, it would seem logial to choose a WT ("normal" yeast strain) as reference, but we still have to choose one among the 48 WT samples. One possiblity is to take a sample that has an average library sum, in order to be in the middle range.

[1] 7125 96

[1] 7125 96

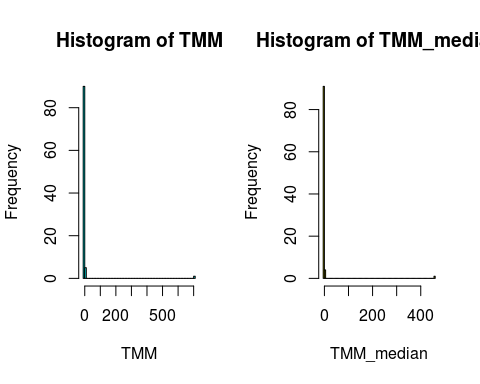
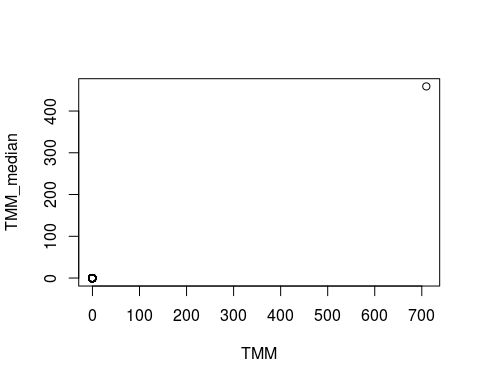
### MA-plot

We will generate various plots to compare the expression level of an arbitrary sample (e.g. sample SNF2.2) with the reference sample (in this case, WT.28).



Count comparisons between 2 arbitrary samples.

On the raw count plot, we see that there is a gene that is highly expressed in the wild-typpe (8e+4 reads) but very weak in the Snf2 mutant (~0 reads)



The Absolute expression level () of gene in samples and is defined as follows.

$$A\_{g} = \frac {1}{2} \log\_{2}(Y\_{gk}/N\_{k} \cdot Y\_{gk}/N\_{k`}) for Y\_{g} \neq 0 $$

Interpretation:

* the value indicates the difference of expression between samples and . A positive sign (resp. negative), indicates that gene is expressed at a higher (resp. lower) level in sample than sample .

We can robustly summarize the observed M values, also we trim both the M values and the A values before taking the weighted average. precision (inverse of the Variance) weights are used to acount for the fact that log fold changes (effectively, a log relative risk) from genes with larger read counts have lower variance on the logarithm scale.

## In practical side to compute TMM for each sample of the 48 replicate data sets.

The Trimmed mean is the average after removing the upper and lower x% of the data. The TMM procedure is doubly trimmed, by log-fold-changes (sample k relative to sample r for gene) and by absolute intensity , by defult we trim the values by 30% and the values by 5%,

### Trimmed Mean of M-values (TMM)

* Motivation total read count is strongly dependent on a few highly expressed transcripts
* Assumption – the majority of genes are not differentially expressed Method – a trimmed mean is the average after removing the upper and lower x% of the data. The TMM procedure is doubly trimmed, by log-fold-changes (sample relative to sample r for gene )
* and by absolute intensity

# The rlog Trasformation

when we need the amount of variance is approximately the same across diffrent mean values,in stady case for multidimentional dta for example clustring and principal components analysis(PCA), this considered common statistical methods for exploratory analysis of multidimentional data. these suppose the data genarally has the same range of variance at diffrent ranges of the mean values this data so-called *HOMOSKEDASTIC*.

## Exercises

### Exercise 1. Summary statistics

Compute a table with summary statistics per samples (columns of the count table). In particular, include in this table the following statistics. All statistics should be computed on both the raw counts and log2-transformed counts.

1. Sum of counts in the sample.
2. Median count per sample.
3. Some illustrative percentiles (0=min, 5, 10, 25, 50, 75, 90, 95, 100=max)
4. Inter-Quartile Range (IQR).
5. Classical statistical estimates (mean, variance, standard deviation).
6. Any other statistics that you moght find relevant.

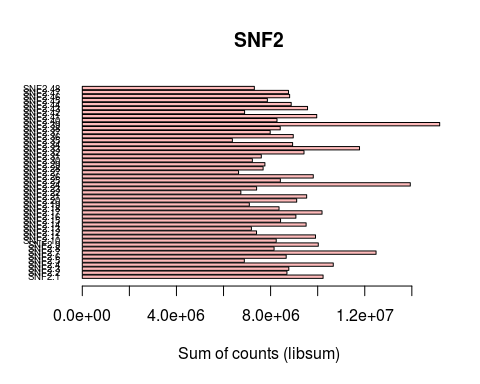
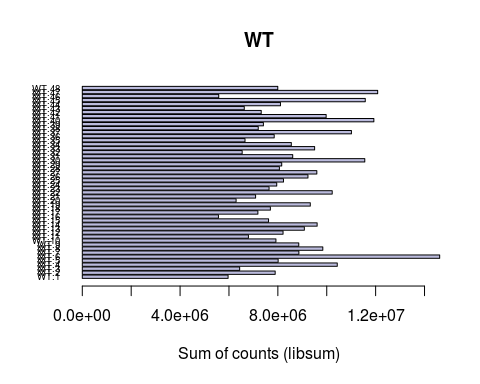
The result table must contain one row per sample (column of the original count table), and one column per statistics (points a to f above).

Hereafter we only display the results for the 10 first samples (columns of the table), for the sake of readibility of the report. However we also calculate the same statistics for each one of the samples (the full table of statistics will be exported in a tab-separated value file).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | WT.1 | WT.2 | WT.3 | WT.4 | WT.5 |
| mean | 8.370686e+02 | 1107.438 | 9.035679e+02 | 1463.891 | 1124.494 |
| sd | 4.439369e+03 | 4814.648 | 4.021288e+03 | 7326.592 | 5272.681 |
| var | 1.970800e+07 | 23180830.837 | 1.617076e+07 | 53678951.273 | 27801168.411 |
| min | 0.000000e+00 | 0.000 | 0.000000e+00 | 0.000 | 0.000 |
| median | 2.240000e+02 | 371.000 | 2.970000e+02 | 430.000 | 353.000 |
| max | 1.888250e+05 | 196804.000 | 1.721190e+05 | 328674.000 | 225435.000 |
| sum | 5.964114e+06 | 7890499.000 | 6.437921e+06 | 10430226.000 | 8012018.000 |
| IQR | 5.120000e+02 | 765.000 | 5.950000e+02 | 914.000 | 735.000 |
| Q0.00 | 0.000000e+00 | 0.000 | 0.000000e+00 | 0.000 | 0.000 |
| Q0.05 | 0.000000e+00 | 0.000 | 0.000000e+00 | 0.000 | 0.000 |
| Q0.10 | 1.000000e+00 | 2.000 | 2.000000e+00 | 2.000 | 2.000 |
| Q0.25 | 4.900000e+01 | 88.000 | 7.500000e+01 | 105.000 | 84.000 |
| Q0.50 | 2.240000e+02 | 371.000 | 2.970000e+02 | 430.000 | 353.000 |
| Q0.75 | 5.610000e+02 | 853.000 | 6.700000e+02 | 1019.000 | 819.000 |
| Q0.90 | 1.472200e+03 | 1918.000 | 1.624200e+03 | 2558.400 | 1898.200 |
| Q0.95 | 2.735200e+03 | 3466.200 | 2.893800e+03 | 4560.000 | 3437.200 |
| Q0.99 | 9.713900e+03 | 13498.000 | 1.072050e+04 | 17262.200 | 14034.100 |
| Q1.00 | 1.888250e+05 | 196804.000 | 1.721190e+05 | 328674.000 | 225435.000 |

### Library size

We will measure the library size for each sample of each genotye, and display the result as boxplot.

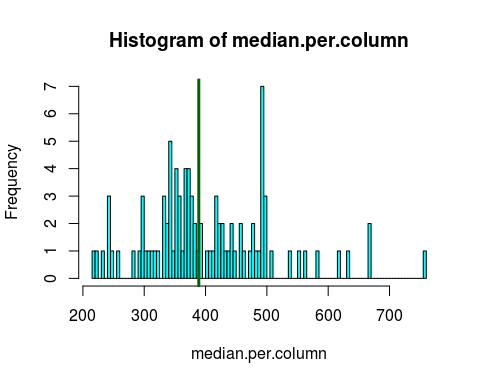


On the barplot, we notice that some libraries have a lower sequencing depth ("libsum") than other ones. For example, sample SNF2.39 totals 15182950 reads whereas SNF2.48 only sums up to 7309429. These two sequencing depths thus differ by a factor two. For this reason, if we want to compute gene-wise statistics, we will need to normalize the counts in order to take into account these sample-wise differences in sequencing depth.

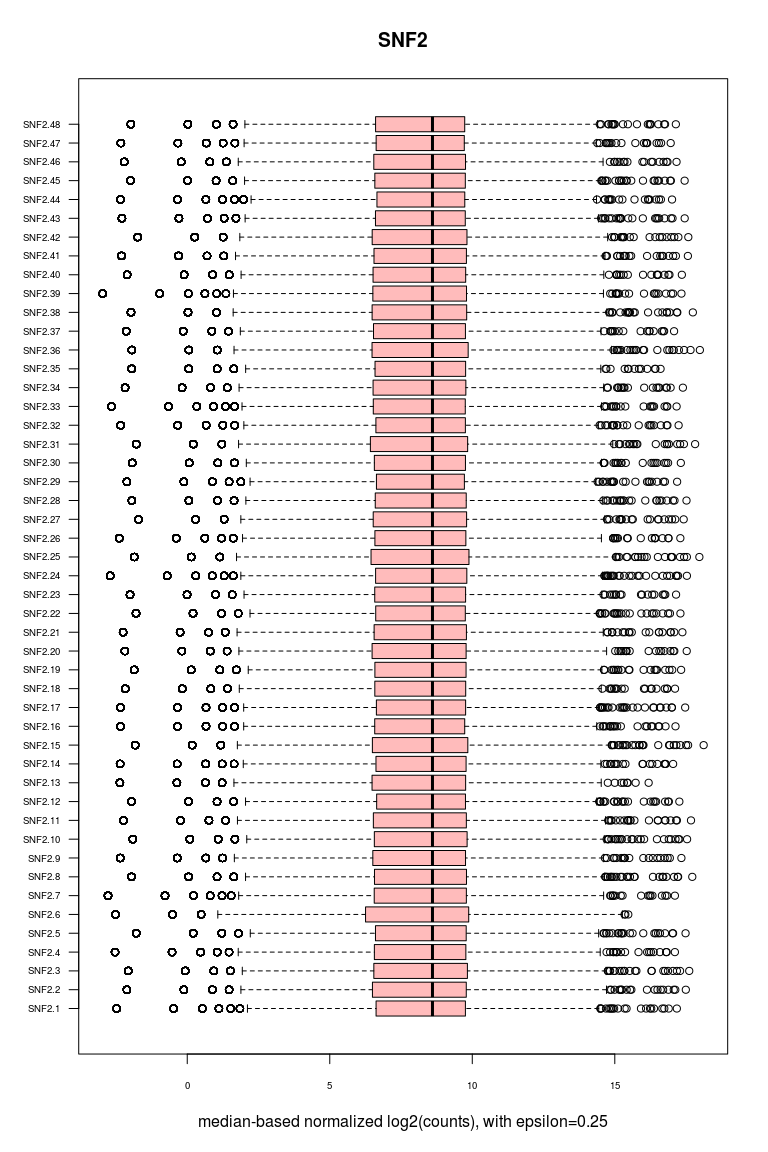
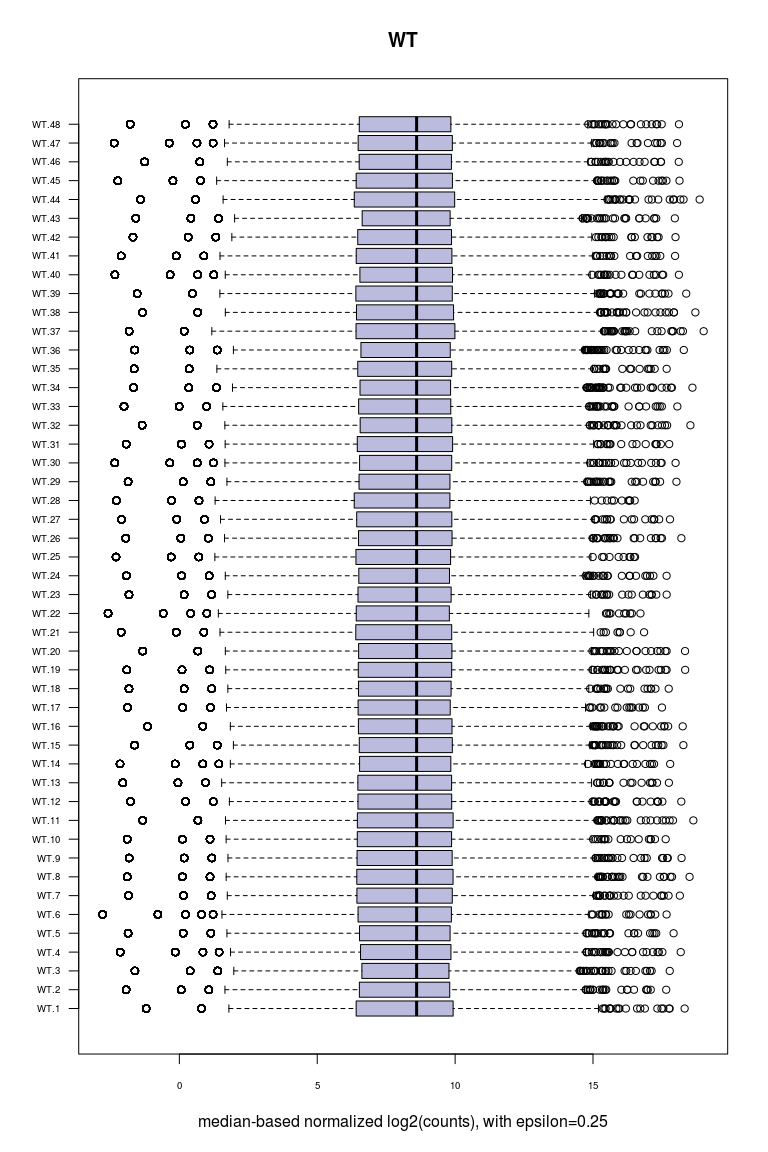
### Exercise 2. Sample-wise standardization

In this exercise we apply a simplistic method to standardize samples. Note that specialized packages like edgeR and DESeq2 have much more elaborate (and relevant) normalization procedures. This simplistic normalization is only for didactic purposes.

1. Standardize the count table by scaling all values accoding to the median per sample (do it for the counts and for the log2-transformed counts).
2. Compare the scaling factors that would be applied with libsum and median-based standardization, respectively (draw a plot comparing the scaling factors for each sample).

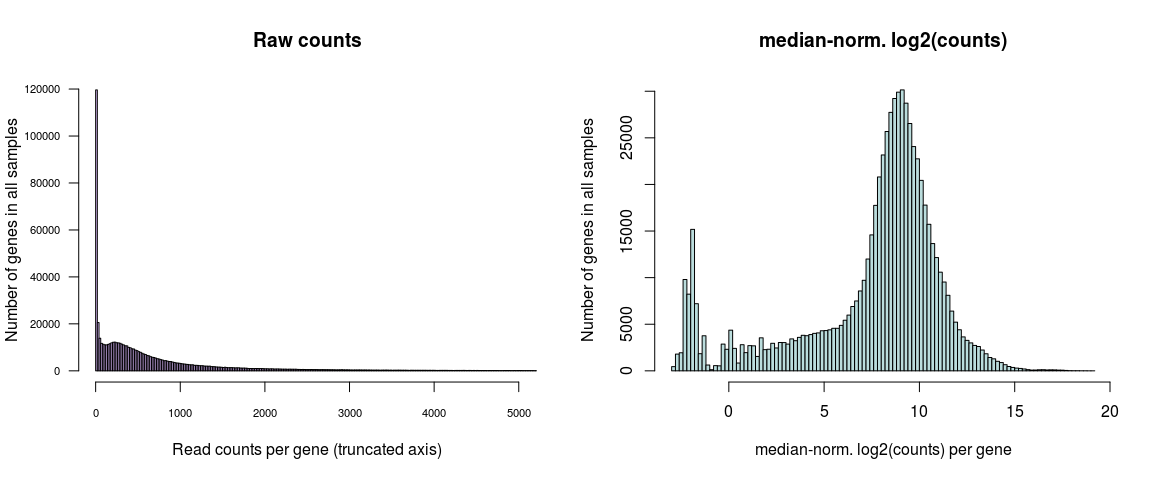
 The boxplots highlight the effect of median-based normalization: after normalization, all samples have the same median.

#### Boxplots of sample-wise standardized counts (log2-transformed)



### Exercise 3. Normalizing effect of the log2 transformation

Plot an histogram of the raw counts (all the table) versus log2-transformed median-based normalized counts.



This distribution does not look like any conventional distribution, but this is not surprizing. Indeed, each gene can be considered as a separate object for which we measure the level of expression in 2 different genotypes (wild-type and Snf2 mutant), and which follows its own distribution, with its own mean and dispersion.

The histograms above can thus be understood as a mixture of 7125 (number of genes) times 2 (number of genotypes) independent distributions, each of which is represented by 48 observations (replicates).

### Exercise 4. Statistics per gene (rows of the count table)

Based on the standardized log2-transformed counts, compute a table with statistics per gene: for each gene, compute the mean, sd, var, min, median, max, IQR, and a few selected quantiles (0, 0.01, 0.05, 0.10, 0.25, 0.50, 0.75, 0.95, 1), ...

\*\*Mustafa: please do the same as what we did above for the samples (columns of the count table), but apply it to genes (rows of the count table).

[1] 7125 10

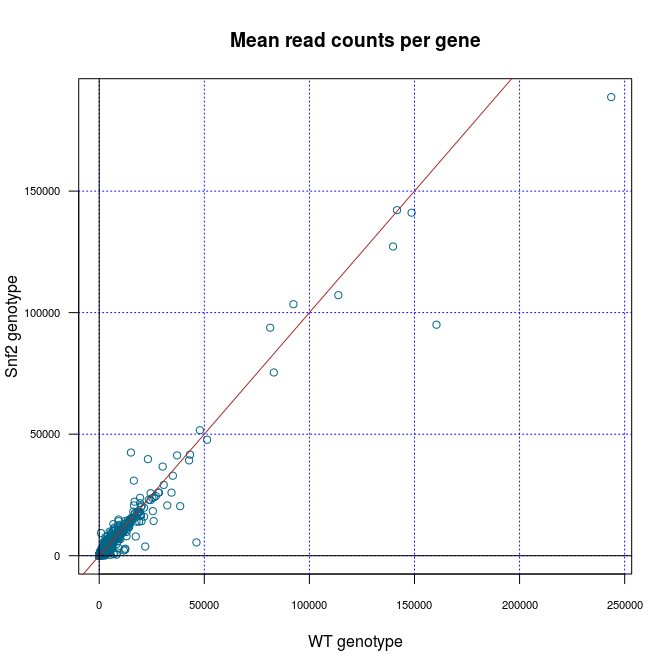
[1] "Q0.00" "Q0.05" "Q0.10" "Q0.25" "Q0.50" "Q0.75" "Q0.90" "Q0.95"  
 [9] "Q0.99" "Q1.00"

[1] "mean" "sd" "var" "min" "median" "max" "IQR"   
 [8] "Q0.00" "Q0.05" "Q0.10" "Q0.25" "Q0.50" "Q0.75" "Q0.90"   
[15] "Q0.95" "Q0.99" "Q1.00"

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 21s\_rrna | hra1 | icr1 | lsr1 | nme1 | pwr1 | q0010 | q0017 | q0032 | q0045 |
| mean | 105.4896 | 2.489583 | 147.98958 | 213.6042 | 27.3125 | 0 | 0 | 0 | 0 | 1.864583 |
| sd | 129.9188 | 1.782930 | 47.74659 | 145.3796 | 15.7696 | 0 | 0 | 0 | 0 | 2.544839 |
| var | 16878.9052 | 3.178838 | 2279.73673 | 21135.2311 | 248.6803 | 0 | 0 | 0 | 0 | 6.476206 |
| min | 10.0000 | 0.000000 | 64.00000 | 55.0000 | 6.0000 | 0 | 0 | 0 | 0 | 0.000000 |
| median | 61.5000 | 2.000000 | 144.00000 | 178.0000 | 24.5000 | 0 | 0 | 0 | 0 | 1.000000 |
| max | 762.0000 | 7.000000 | 286.00000 | 895.0000 | 115.0000 | 0 | 0 | 0 | 0 | 13.000000 |
| IQR | 74.7500 | 3.000000 | 69.50000 | 124.5000 | 16.2500 | 0 | 0 | 0 | 0 | 3.000000 |
| Q0.00 | 10.0000 | 0.000000 | 64.00000 | 55.0000 | 6.0000 | 0 | 0 | 0 | 0 | 0.000000 |
| Q0.05 | 17.2000 | 0.000000 | 84.50000 | 65.8000 | 9.8000 | 0 | 0 | 0 | 0 | 0.000000 |
| Q0.10 | 22.0000 | 0.000000 | 92.50000 | 85.0000 | 11.5000 | 0 | 0 | 0 | 0 | 0.000000 |
| Q0.25 | 32.8000 | 1.000000 | 113.80000 | 127.2000 | 17.8000 | 0 | 0 | 0 | 0 | 0.000000 |
| Q0.50 | 61.5000 | 2.000000 | 144.00000 | 178.0000 | 24.5000 | 0 | 0 | 0 | 0 | 1.000000 |
| Q0.75 | 107.5000 | 4.000000 | 183.20000 | 251.8000 | 34.0000 | 0 | 0 | 0 | 0 | 3.000000 |
| Q0.90 | 247.5000 | 5.000000 | 209.00000 | 393.0000 | 40.5000 | 0 | 0 | 0 | 0 | 5.000000 |
| Q0.95 | 364.8000 | 5.000000 | 240.50000 | 477.2000 | 52.0000 | 0 | 0 | 0 | 0 | 6.200000 |
| Q0.99 | 588.1000 | 6.000000 | 275.50000 | 749.6000 | 81.7000 | 0 | 0 | 0 | 0 | 12.000000 |
| Q1.00 | 762.0000 | 7.000000 | 286.00000 | 895.0000 | 115.0000 | 0 | 0 | 0 | 0 | 13.000000 |

### Exercise 5. Comparison between WT and mutant genotypes

1. Compute the mean counts per gene for each genotype, using the median-based standardized log2-transformed counts.
2. Draw a plot to compare the mean expression per gene between WT and SNF2 genotypes.

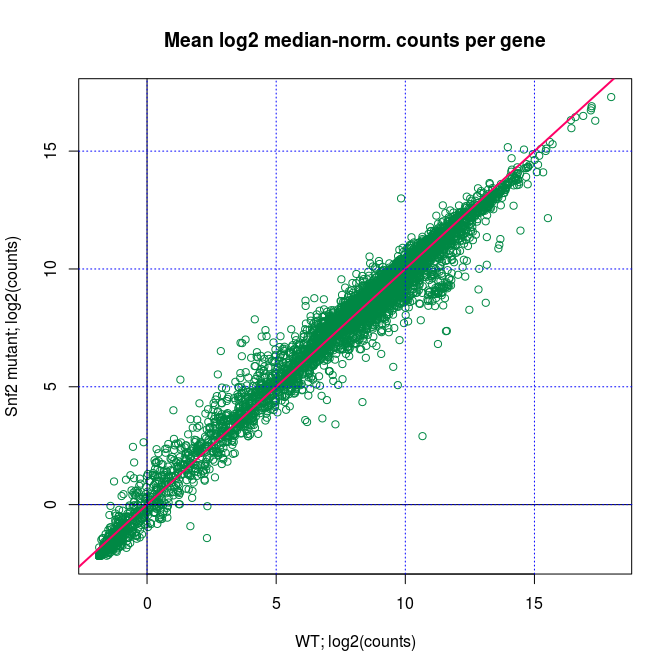


Comparison of mean read counts per gene between the two genotypes. Note that this drawing is very preliminary, since we did not yet normalize the libraries. It is just to get a rough idea.

On this plot, each dot corresponds to one gene, and its horizontal and vertical position indicate its mean raw counts for WT and Snf2 samples, respectively.

### Log2 mean values

We can also draw a plot with the mean of the log2-transformed counts. Note that the arithmetic mean of the logarithms is equivalent to the logarithm of the geometric mean.



Comparison of mean read log2(counts) per gene between the two genotypes. Note that this drawing is very preliminary, since we did not yet normalize the libraries. It is just to get a rough idea.

## Next steps

1. Do the exercises above (exploration of the data)
2. Learn to use the *DESeq2* and *edgeR* packages by using the Vignettes.
3. Run DESeq2 and edgeR on the yeast dataset, in order to detect differentially expressed genes between WT and Snf2.
4. After that, you will analyse the robustness of the lists of differentially expressed genes by running resampling approaches (bootstrap, jacknife, subsampling).

We will now create a DGEList object to hold our read counts. DGEList is an example of the custom task-specific structure that are frequently used in Bioconductor to make analyses easier. **DGEList()** is the function that converts the count matrix into an edgeR object. BUT Firstly:- we must have a group variables that tells edgeR which samples belong to which group.

[1] 20 3 75 60 13

[1] "21s\_rrna" "hra1" "icr1" "lsr1" "nme1"

$names  
[1] "counts" "samples" "genes"   
  
$class  
[1] "DGEList"  
attr(,"package")  
[1] "edgeR"

WT.1 WT.2 WT.3 WT.4 WT.5  
ydl160c 706 622 579 995 640  
ydl160c-a 124 183 161 269 174  
ydl161w 1084 1086 935 1786 1219  
ydl162c 4 2 3 0 3  
ydl163w 0 0 0 0 0  
ydl164c 280 387 301 487 387  
ydl165w 229 358 267 491 326  
ydl166c 359 620 408 674 784  
ydl167c 219 603 217 384 682  
ydl168w 646 841 608 976 747  
ydl169c 131 121 181 235 106

WT.1 WT.2 WT.3 WT.4 WT.5  
21s\_rrna 20 76 101 99 128  
hra1 3 2 2 2 3  
icr1 75 123 107 157 98  
lsr1 60 163 233 163 193  
nme1 13 14 23 13 29  
pwr1 0 0 0 0 0  
q0010 0 0 0 0 0  
q0017 0 0 0 0 0  
q0032 0 0 0 0 0  
q0045 0 2 1 2 2

[1] 7125 96

[1] 7125 96

[1] 7125 96

### Filtering out undetected genes

#### Why we will do filtering out undetected genes?

in view of these genes could not adequately represented or not have enough reads to contribute to analysis. in addition that the low count reads it would be impossible to detecte differential expression.

**Motivation:**  now we will apply a filter to discard genes that remain undetected in almost all the samples.

**Explanation:**  In our dataset we have approximatly 7125 Genes. However, many of them will not be expressed, or will not be represented by enough reads to contribute to the analysis. Removing these genes means that we ultimately have fewer tests to perform, thereby we will reduce the problems associated with multiple testing by using **cpm** (= **Counts per million**), whereas we will retain only those genes that are repersented at least 1 cpm reads in our two groups for gene "WT" and "SNF2" (MAEQ, Summarized from[[1]](#footnote-76)).

#### How we will do filter out the low count reads?

By keep only those genes that have at least 1 or 2 read per million in at least 96 samples.and thence we can calcluate the Nomalization factors which correct for the different composition of the samples. the effective Library sizes are then the product of actual Library sizes and these factors.

**Procedure:**  starting from the raw count table,

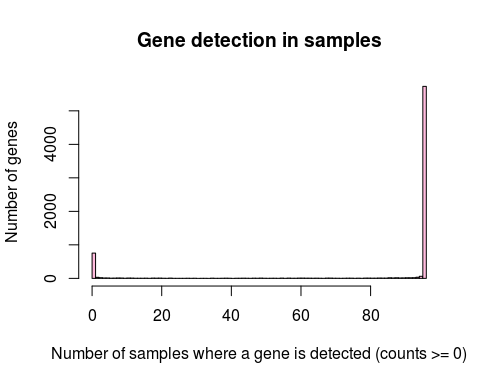
1. We compute a Boolean table named countcheck, which indicates whether each gene (rows) is or not detected in each sample (column). Our criterion for considering a gene as detected or not is to have at least one count for this gene in this sample.
2. Compute the marginal sums on each row of the Boolean countcheck table, and store it in a Boolean vector named countcheck.sum, which indicates, for each gene, the number of samples in which it has been detected.
3. We plot an histogram showing the distribution of the number of samples where each gene is detected.
4. We compute a Boolean vector indicating whether each gene has been detected in at least 2 samples. We discard all the genes that fail to pass this criterion.

[1] "Min. : 0.00 " "1st Qu.: 8.22 " "Median : 37.56 "  
 [4] "Mean : 140.35 " "3rd Qu.: 94.06 " "Max. :31660.19 "  
 [7] "Min. : 0.00 " "1st Qu.: 11.15 " "Median : 47.02 "  
[10] "Mean : 140.35 "

[1] "Total number of values = 684000"

[1] "Number of values > 1CPM: 586808"

[1] "Percent of values > 1CPM: 85.8%"



keep.Boolean  
FALSE TRUE   
 755 6370

21s\_rrna icr1 lsr1 nme1 q0045 q0050 q0055 q0130   
 1 3 4 5 10 11 12 24   
 q0160 q0275   
 29 33

[1] "Initial number of genes: 7125"

[1] "Number of kept genes: 6370"

[1] "Number of kept genes: 6370"

[1] "Percent of kept genes: 89.4"

An object of class "DGEList"  
$counts  
 WT.1 WT.2 WT.3 WT.4 WT.5 WT.6 WT.7 WT.8 WT.9 WT.10 WT.11 WT.12  
21s\_rrna 20 76 101 99 128 74 36 32 57 104 194 11  
icr1 75 123 107 157 98 245 119 120 119 132 91 98  
lsr1 60 163 233 163 193 375 194 84 211 158 65 138  
nme1 13 14 23 13 29 40 28 23 26 22 9 11  
q0045 0 2 1 2 2 0 0 1 0 1 1 0  
 WT.13 WT.14 WT.15 WT.16 WT.17 WT.18 WT.19 WT.20 WT.21 WT.22 WT.23  
21s\_rrna 40 75 20 221 119 270 89 261 27 158 109  
icr1 126 156 114 70 129 134 118 89 158 197 115  
lsr1 199 131 99 314 206 411 116 465 194 424 226  
nme1 23 9 15 33 22 44 14 32 12 37 38  
q0045 1 1 1 2 1 5 0 3 0 0 1  
 WT.24 WT.25 WT.26 WT.27 WT.28 WT.29 WT.30 WT.31 WT.32 WT.33 WT.34  
21s\_rrna 46 63 352 54 57 31 61 34 76 110 101  
icr1 116 139 111 133 143 104 186 133 83 112 86  
lsr1 139 277 401 201 209 153 177 265 80 264 263  
nme1 24 24 37 25 23 18 22 34 19 44 26  
q0045 0 1 1 0 0 0 1 0 2 2 2  
 WT.35 WT.36 WT.37 WT.38 WT.39 WT.40 WT.41 WT.42 WT.43 WT.44 WT.45  
21s\_rrna 86 193 29 71 10 274 49 30 72 58 21  
icr1 95 85 111 64 85 177 137 118 113 81 142  
lsr1 223 223 100 55 66 385 232 149 114 81 109  
nme1 20 22 20 13 6 52 31 22 10 15 23  
q0045 0 1 0 0 0 5 1 2 0 1 0  
 WT.46 WT.47 WT.48 SNF2.1 SNF2.2 SNF2.3 SNF2.4 SNF2.5 SNF2.6  
21s\_rrna 159 107 70 31 18 23 44 356 62  
icr1 94 187 106 205 196 211 252 127 146  
lsr1 132 243 128 210 103 159 260 298 522  
nme1 10 40 17 32 20 17 41 35 7  
q0045 1 8 1 0 5 0 4 4 2  
 SNF2.7 SNF2.8 SNF2.9 SNF2.10 SNF2.11 SNF2.12 SNF2.13 SNF2.14  
21s\_rrna 35 33 13 148 64 23 15 762  
icr1 275 160 190 146 191 162 145 207  
lsr1 303 96 132 263 157 178 239 895  
nme1 37 20 16 45 30 30 37 115  
q0045 0 4 0 4 5 0 0 13  
 SNF2.15 SNF2.16 SNF2.17 SNF2.18 SNF2.19 SNF2.20 SNF2.21 SNF2.22  
21s\_rrna 39 38 391 82 58 29 88 55  
icr1 145 175 203 178 131 157 188 148  
lsr1 60 137 439 178 121 144 257 125  
nme1 14 34 52 32 29 36 26 14  
q0045 3 0 7 0 0 1 3 0  
 SNF2.23 SNF2.24 SNF2.25 SNF2.26 SNF2.27 SNF2.28 SNF2.29 SNF2.30  
21s\_rrna 39 433 139 74 51 192 60 170  
icr1 157 264 105 215 98 151 216 123  
lsr1 175 514 90 153 102 199 134 265  
nme1 17 57 24 40 11 28 26 30  
q0045 3 5 2 3 2 1 1 6  
 SNF2.31 SNF2.32 SNF2.33 SNF2.34 SNF2.35 SNF2.36 SNF2.37 SNF2.38  
21s\_rrna 45 28 76 45 579 30 30 18  
icr1 102 218 239 152 140 116 145 149  
lsr1 81 128 306 189 713 86 168 108  
nme1 10 26 28 37 71 9 22 25  
q0045 0 2 1 1 11 1 3 0  
 SNF2.39 SNF2.40 SNF2.41 SNF2.42 SNF2.43 SNF2.44 SNF2.45 SNF2.46  
21s\_rrna 64 79 67 13 576 52 234 23  
icr1 286 155 184 164 202 186 148 183  
lsr1 250 195 153 57 742 227 227 117  
nme1 38 18 26 18 80 34 26 23  
q0045 4 0 1 0 12 3 4 0  
 SNF2.47 SNF2.48  
21s\_rrna 44 24  
icr1 195 145  
lsr1 224 136  
nme1 24 28  
q0045 3 1  
6365 more rows ...  
  
$samples  
 group lib.size norm.factors  
WT.1 1 5964114 1  
WT.2 1 7890499 1  
WT.3 1 6437921 1  
WT.4 1 10430226 1  
WT.5 1 8012018 1  
91 more rows ...  
  
$genes  
[1] "21s\_rrna" "icr1" "lsr1" "nme1" "q0045"   
6365 more rows ...

[1] "Min. : 0.00 " "1st Qu.: 17.61 " "Median : 45.10 "  
 [4] "Mean : 156.98 " "3rd Qu.: 107.27 " "Max. :31660.19 "  
 [7] "Min. : 0.00 " "1st Qu.: 23.70 " "Median : 55.83 "  
[10] "Mean : 156.98 "

#### What ?

What do we observe ? What do we conclude (answer the original question of the motivation) ?

\*Interpretation of the result: \*\* Among the XXX genes in our orginal count table, XXX (YYY%) were filtered out because they wer detected in less than 2 samples.

## NORMALISATION by unsing edgeR package

It is important to Normalise RNA-Seq both within and between samples, edgeR implements the trimmed mean of M-Values (TMM) method.

WT.1 WT.2 WT.3 WT.4 WT.5 WT.6 WT.7 WT.8 WT.9  
21s\_rrna 20 76 101 99 128 74 36 32 57  
icr1 75 123 107 157 98 245 119 120 119  
lsr1 60 163 233 163 193 375 194 84 211  
nme1 13 14 23 13 29 40 28 23 26  
q0045 0 2 1 2 2 0 0 1 0  
q0050 2 1 6 6 14 6 2 1 5  
q0055 0 5 4 1 11 8 3 1 2  
q0130 1 1 1 1 3 4 5 1 3  
q0160 0 0 0 0 2 2 1 1 0  
q0275 0 1 2 0 3 1 0 0 1  
rdn5-1 50 42 105 48 36 80 37 50 49  
rdn5-2 3 1 2 6 3 9 8 7 3  
rdn5-6 21 11 71 7 16 15 16 25 23  
rna170 8 10 8 13 5 19 21 13 18  
rpr1 31 42 51 93 79 83 80 48 145  
ruf20 38 146 148 136 129 240 88 55 92  
ruf21 5 50 43 36 53 105 21 5 9  
ruf23 1 8 7 5 8 11 5 6 9  
ruf5-1 159 404 313 454 457 732 297 155 247  
ruf5-2 101 304 211 251 321 465 172 67 164  
scr1 143 165 468 194 380 304 234 163 453  
srg1 97 278 301 388 283 566 221 171 226  
tlc1 729 1510 1167 1717 1455 2939 1191 1033 1130  
yal001c 311 421 382 550 403 929 499 414 379  
yal002w 266 451 434 616 441 913 503 431 376  
yal003w 6387 10292 7440 12994 11001 16296 10198 10137 11298  
yal005c 10331 10088 10932 19029 9711 18561 12207 16760 12184  
yal007c 596 895 787 1180 836 1682 948 890 811  
yal008w 278 357 346 596 290 591 317 392 431  
yal009w 205 291 238 371 251 570 285 297 298  
yal010c 179 341 281 423 262 581 250 268 316  
yal011w 216 383 288 397 375 759 369 355 309  
yal012w 4251 3737 3007 5536 4111 8171 5270 5949 4895  
yal013w 776 962 717 1097 1062 1913 1020 1388 1338  
yal014c 840 923 717 1136 939 1592 962 1222 1279  
yal015c 318 346 291 490 347 577 368 434 394  
yal016c-a 17 29 24 30 19 35 17 40 29  
yal016c-b 21 33 43 54 15 72 31 23 23  
yal016w 999 1551 1318 1991 1419 2826 1551 1703 1448  
yal017w 855 922 882 1334 819 2086 1017 1258 862  
yal018c 0 1 2 6 7 5 1 1 1  
yal019w 368 781 387 628 859 1768 860 675 676  
yal020c 319 359 274 529 392 582 415 531 459  
yal021c 802 1125 847 1367 1067 1914 1134 1428 1288  
yal022c 793 855 608 1054 939 1592 979 1407 1079  
yal023c 2588 3218 2316 3948 3307 6413 3802 4172 3815  
yal024c 122 255 172 220 210 494 209 207 206  
yal025c 369 1108 520 664 1302 2505 1007 921 1010  
yal026c 512 833 579 850 784 1694 926 907 757  
yal026c-a 6 27 24 31 18 63 6 5 9  
yal027w 146 229 201 303 253 417 218 238 236  
yal028w 340 348 308 504 313 609 375 521 351  
yal029c 664 1246 890 1251 1197 2576 1274 1182 1024  
yal030w 449 560 540 886 473 770 472 674 601  
yal031c 276 451 405 529 449 834 418 459 421  
yal031w-a 5 7 10 7 3 19 7 3 1  
yal032c 375 515 370 642 467 841 461 576 493  
yal033w 158 268 236 283 281 405 235 297 220  
yal034c 354 463 442 715 382 811 410 614 494  
yal034c-b 21 42 30 42 33 86 24 17 25  
yal034w-a 78 116 103 113 113 206 109 112 96  
yal035w 2815 4160 2969 4411 4836 9788 5245 5191 4345  
yal036c 331 599 348 525 691 1278 766 756 665  
yal037c-a 0 37 23 15 51 164 22 10 14  
yal037c-b 70 183 199 208 196 479 289 102 86  
yal037w 22 69 32 58 51 118 58 37 52  
yal038w 21080 18869 14649 28551 20208 38816 35125 38974 29292  
yal039c 1541 1447 1318 2251 1501 2590 1725 2339 2329  
yal040c 373 944 450 773 847 1901 794 701 712  
yal041w 413 636 423 704 653 1232 721 689 592  
yal042w 2661 2758 2313 3914 2852 5485 3452 4155 3856  
yal043c 597 712 569 974 780 1380 845 987 924  
yal044c 1486 1027 1013 2058 1100 1784 1216 1794 1447  
yal044w-a 33 36 37 62 32 51 43 35 39  
yal046c 209 233 194 315 255 392 232 376 289  
yal047c 107 189 143 232 201 355 177 160 180  
yal048c 133 129 98 188 134 298 178 183 154  
yal049c 448 562 461 751 502 1000 516 711 617  
yal051w 256 496 362 557 459 977 426 459 424  
yal053w 2489 2567 2387 4560 2297 4830 3395 3631 3340  
yal054c 198 256 243 343 250 429 262 357 309  
yal055w 279 278 256 407 300 466 277 451 400  
yal056c-a 1 3 4 2 7 7 8 1 6  
yal056w 697 1125 823 1177 1079 2114 1073 1172 1108  
yal058w 222 228 191 305 237 457 271 303 260  
yal059w 41 163 102 116 183 412 149 93 106  
yal060w 1401 1210 1345 2356 1133 2031 1363 2260 1689  
yal061w 2114 1725 1485 3013 1544 2846 1915 2976 2486  
yal062w 389 401 370 534 343 754 438 701 488  
yal063c 236 255 194 343 267 541 325 428 316  
yal063c-a 1 6 6 9 7 10 0 3 2  
yal064c-a 35 43 38 48 28 59 42 65 59  
yal064w 1 5 5 9 4 5 2 4 3  
yal064w-b 16 25 19 19 22 40 29 36 32  
yal065c 24 27 22 33 25 51 37 45 28  
yal067c 11 23 20 28 29 37 19 23 18  
yal068c 4 6 6 13 8 17 8 17 20  
yar002c-a 763 1351 1227 1795 1332 2426 1334 1338 1218  
yar002w 413 815 587 793 823 1745 826 684 765  
yar003w 300 472 373 519 486 799 491 452 424  
yar007c 440 475 539 757 514 915 506 591 473  
yar008w 109 226 163 217 188 421 187 194 172  
yar009c 1242 1708 1405 2207 1834 4065 2108 2185 1655  
yar010c 403 608 442 807 530 1243 754 801 581  
yar014c 648 887 628 947 915 1831 1028 1045 1007  
yar015w 2542 1805 1652 4246 2038 3186 2365 1911 2441  
yar018c 185 289 233 352 287 513 237 293 254  
yar019c 173 322 228 361 250 544 287 295 266  
yar019w-a 4 4 1 1 6 5 0 2 8  
yar020c 28 28 35 42 29 62 30 34 33  
yar023c 63 79 82 134 76 125 79 88 107  
yar027w 549 711 676 1460 651 1203 842 632 839  
yar028w 228 288 203 508 288 563 429 286 401  
yar029w 7 26 18 25 31 54 25 15 17  
yar030c 15 23 18 32 36 61 20 16 28  
yar031w 107 171 144 277 150 322 193 170 169  
yar033w 170 283 226 372 227 444 233 290 252  
yar035c-a 1 0 2 1 2 4 1 2 0  
yar035w 193 132 144 221 146 242 156 395 203  
yar042w 1008 1098 861 1346 939 2108 1234 1501 1257  
yar047c 10 17 11 10 8 21 6 7 5  
yar050w 237 228 172 261 252 539 334 404 259  
yar053w 2 7 2 8 11 13 1 12 5  
yar060c 0 5 4 1 5 8 4 2 2  
yar061w 0 3 0 4 4 16 6 6 2  
yar062w 12 9 9 14 10 23 11 15 2  
yar064w 13 17 10 17 13 40 29 32 30  
yar066w 36 52 33 55 41 83 59 67 59  
yar068w 119 91 90 136 99 190 122 237 117  
yar070c 0 7 4 3 4 10 4 4 3  
yar071w 2020 2554 1888 2934 2916 5002 3405 3437 3151  
yar073w 214 291 237 429 292 583 468 315 346  
yar075w 143 144 124 249 185 333 228 213 163  
ybl001c 559 741 746 1158 663 1139 729 882 740  
ybl002w 1175 2838 2234 3158 2293 4577 2277 1993 2857  
ybl003c 2021 4142 3524 5169 3883 7155 3897 3664 3817  
ybl004w 528 1514 834 1177 1563 3535 1293 1123 1036  
ybl005w 395 737 607 771 759 1544 669 665 570  
ybl005w-b 1399 1894 1487 2379 2024 4398 2416 2406 1700  
ybl006c 605 744 613 1009 661 1052 692 1054 957  
ybl007c 2232 2241 1928 3262 2147 4585 2757 3398 2630  
ybl008w 143 301 205 336 345 505 294 289 252  
ybl009w 140 359 196 272 309 651 313 246 241  
ybl010c 106 156 120 167 170 311 158 141 160  
ybl011w 866 1301 995 1446 1232 2511 1285 1468 1351  
ybl012c 4 13 16 13 8 30 7 6 14  
ybl013w 39 54 39 35 39 83 45 65 58  
ybl014c 127 268 144 214 312 643 288 181 191  
ybl015w 732 619 599 981 553 1154 858 1136 752  
ybl016w 309 523 357 561 642 975 547 597 633  
ybl017c 1613 1878 1565 2912 1695 3947 2325 2221 1837  
ybl018c 174 238 188 295 323 406 257 313 306  
ybl019w 117 183 166 225 170 350 164 156 190  
ybl020w 258 511 348 482 478 876 466 384 425  
ybl021c 248 287 246 364 326 529 286 431 412  
ybl022c 1168 1466 1364 1942 1428 3122 1550 1861 1351  
ybl023c 331 511 379 575 495 1007 484 543 500  
ybl024w 451 1177 715 948 1087 2057 1025 786 834  
ybl025w 64 131 114 162 132 258 126 130 149  
ybl026w 206 482 394 579 474 737 372 355 382  
ybl027w 9723 14748 11264 18152 16124 26609 15844 17139 14440  
ybl028c 77 378 202 219 363 773 247 163 210  
ybl029c-a 157 245 234 363 182 326 193 206 259  
ybl029w 189 303 278 398 282 458 232 264 282  
ybl030c 2578 2664 2887 4014 2639 5255 3226 3877 3212  
ybl031w 95 236 178 267 262 361 170 170 231  
ybl032w 916 1271 1094 1539 1310 2168 1292 1461 1417  
ybl033c 967 869 851 1369 849 1213 926 1408 1159  
ybl034c 126 246 178 242 215 485 240 203 172  
ybl035c 118 175 152 227 164 344 169 171 121  
ybl036c 280 518 417 587 552 1069 526 460 477  
ybl037w 384 444 424 607 452 899 537 531 419  
ybl038w 293 449 462 614 502 837 441 414 446  
ybl039c 428 1075 525 782 1207 2428 1302 996 1016  
ybl039w-b 43 90 100 143 88 159 70 66 70  
ybl040c 418 878 698 1158 738 1159 665 719 749  
ybl041w 1954 2486 2162 3277 2361 4047 2363 3095 3027  
ybl042c 336 416 283 525 396 832 492 451 444  
ybl043w 53 69 73 109 53 109 86 106 91  
ybl044w 10 20 11 17 8 13 8 10 18  
ybl045c 1421 1335 1589 2285 1235 2692 1802 1915 1663  
ybl046w 531 672 514 791 647 1155 692 883 926  
ybl047c 3001 2916 2616 4259 2788 6260 3661 4382 3307  
ybl048w 10 20 14 27 21 38 28 9 17  
ybl049w 49 86 71 116 86 141 83 86 61  
ybl050w 647 1084 1038 1472 960 1818 968 984 981  
ybl051c 894 1258 847 1467 1200 2585 1337 1316 1307  
ybl052c 141 373 289 342 367 755 331 300 243  
ybl053w 2 6 5 12 6 13 1 1 0  
ybl054w 124 397 145 161 427 783 303 266 248  
ybl055c 485 698 507 884 650 1308 847 809 702  
ybl056w 1915 2356 2202 3300 2447 4299 2683 3200 2646  
ybl057c 567 802 614 982 879 1149 746 1005 952  
ybl058w 1628 2072 1941 2824 2113 3987 2131 2584 2495  
ybl059c-a 108 209 179 238 171 280 152 187 161  
ybl059w 89 133 152 182 147 213 121 155 124  
ybl060w 149 304 235 341 279 547 243 310 248  
ybl061c 481 698 542 682 655 1270 689 882 643  
ybl063w 148 305 262 305 299 617 280 250 262  
ybl064c 719 888 1049 1434 815 1343 757 1247 1033  
ybl065w 0 3 1 3 1 4 2 1 1  
ybl066c 195 301 186 316 217 584 298 263 263  
ybl067c 412 777 600 926 719 1415 679 605 563  
ybl068w 274 592 387 576 627 1121 549 564 534  
ybl069w 189 244 158 295 253 449 282 255 289  
ybl070c 1 6 3 5 8 9 3 2 3  
ybl071c 27 87 45 65 80 135 47 40 48  
ybl071c-b 1 2 3 7 7 6 2 1 4  
ybl071w-a 49 109 66 106 135 147 68 77 97  
ybl072c 9601 16257 12344 19746 16616 29653 17186 16518 15840  
ybl074c 42 64 44 73 64 129 62 84 57  
ybl075c 223 362 268 357 387 667 278 321 322  
ybl076c 3055 4234 2886 4751 4263 9067 5464 4779 4313  
ybl077w 22 47 46 54 60 106 59 21 19  
ybl078c 210 323 359 525 299 459 315 367 435  
ybl079w 406 774 558 766 751 1694 833 683 575  
ybl080c 180 304 218 337 268 426 251 313 270  
ybl081w 342 850 467 664 973 1763 760 714 895  
ybl082c 434 477 309 526 473 890 606 717 638  
ybl083c 1 5 0 4 3 4 3 2 1  
ybl084c 214 366 282 418 301 586 302 364 335  
ybl085w 762 1388 1069 1644 1320 2757 1410 1359 1198  
ybl086c 556 854 689 1062 786 1552 753 892 887  
ybl087c 5221 7425 5470 9395 8282 12125 8206 8628 9057  
ybl088c 335 548 466 729 473 1071 502 463 476  
ybl089w 236 354 309 435 341 591 302 388 345  
ybl090w 133 306 289 392 277 515 243 254 284  
ybl091c 2267 2619 2251 3748 2707 4251 2640 3459 3640  
ybl091c-a 318 347 332 574 328 525 315 456 457  
ybl092w 11110 17673 13084 21802 19361 27998 18330 20728 18926  
ybl093c 574 1009 668 933 1029 1754 906 988 1031  
ybl095w 97 116 113 171 130 200 126 180 158  
ybl096c 1 3 4 3 5 12 4 8 3  
ybl097w 94 117 81 132 137 343 139 157 115  
ybl098w 129 174 145 214 160 314 166 159 156  
ybl099w 2570 2925 2932 4513 2785 5324 3555 3957 3305  
ybl100c 14 45 33 42 37 82 44 21 45  
ybl100w-b 344 560 473 686 556 1194 625 613 457  
ybl100w-c 1 4 0 6 2 5 5 2 2  
ybl101c 1213 706 694 1484 653 1409 872 1198 761  
ybl102w 494 624 597 977 600 1077 724 808 745  
ybl103c 402 462 404 686 455 932 532 518 444  
ybl104c 221 369 234 420 390 758 419 414 361  
ybl105c 966 1344 1137 1637 1342 2971 1558 1352 1380  
ybl106c 193 240 166 296 227 542 272 259 213  
ybl107c 240 422 398 519 411 752 359 356 370  
ybl107w-a 8 16 11 6 14 29 4 3 6  
ybl108c-a 9 13 17 23 16 28 18 18 10  
ybl108w 0 1 4 1 1 4 1 1 2  
ybl109w 2 0 3 7 4 18 6 3 3  
ybl111c 139 152 86 144 148 306 211 250 157  
ybl112c 44 41 24 37 42 61 42 66 48  
ybl113c 717 580 489 913 686 1165 765 1210 886  
ybr001c 493 656 644 892 603 1196 611 734 694  
ybr002c 288 499 307 448 505 916 481 413 517  
ybr003w 479 718 632 864 637 1258 685 797 713  
ybr004c 242 689 439 754 525 1110 571 406 521  
ybr005w 755 869 540 1864 589 1313 1270 954 1393  
ybr006w 359 383 390 763 332 695 492 413 460  
ybr007c 212 443 336 456 399 818 372 375 354  
ybr008c 180 408 272 376 371 732 334 333 323  
ybr009c 3065 5750 4668 6034 5770 9506 4651 6444 6493  
ybr010w 1974 5429 4352 5431 4896 8345 4155 4135 4434  
ybr011c 5883 8163 6712 11144 8802 12855 7698 9354 9795  
ybr012c 30 46 57 65 57 107 65 38 33  
ybr012w-b 1150 1603 1288 1986 1670 3471 1924 1957 1431  
ybr013c 39 74 72 126 67 131 41 64 67  
ybr014c 233 433 407 490 347 700 368 384 396  
ybr015c 1239 1635 1308 1954 1678 3106 1727 1987 1785  
ybr016w 1110 1265 1179 1987 1184 2209 1258 1766 1609  
ybr017c 678 1242 781 1191 1227 2492 1262 1256 1204  
ybr018c 27 61 30 58 42 81 38 46 42  
ybr019c 60 115 112 176 110 193 93 145 126  
ybr020w 75 150 112 141 133 257 126 118 106  
ybr021w 128 217 156 192 186 405 185 165 213  
ybr022w 143 226 183 285 203 315 222 264 228  
ybr023c 854 1317 1133 1519 1214 2459 1297 1419 1203  
ybr024w 151 220 209 332 245 470 264 268 252  
ybr025c 3607 6243 4516 6882 6184 11171 6508 6153 6141  
ybr026c 483 674 643 1034 556 1069 587 782 693  
ybr027c 15 23 26 25 20 50 15 9 14  
ybr028c 295 489 429 500 503 927 427 369 414  
ybr029c 455 896 653 990 932 1587 815 739 815  
ybr030w 204 337 258 353 459 842 390 356 392  
ybr031w 18186 21323 16627 28517 21976 42762 25694 30143 26495  
ybr032w 44 174 134 94 202 335 118 138 170  
ybr033w 113 252 184 257 232 391 192 254 202  
ybr034c 703 1768 962 1181 1968 3203 1643 1545 1784  
ybr035c 725 817 804 1305 808 1554 891 889 891  
ybr036c 2126 2187 1827 3400 1978 3879 2816 3159 2966  
ybr037c 340 366 370 532 333 647 357 474 399  
ybr038w 406 574 460 562 544 1069 539 507 501  
ybr039w 1046 1220 1207 1835 1115 2301 1455 1414 1396  
ybr041w 703 990 833 1322 806 1680 1056 1067 945  
ybr042c 284 514 443 605 518 946 551 455 438  
ybr043c 421 563 402 690 505 1070 620 549 537  
ybr044c 315 485 442 626 489 939 492 518 495  
ybr045c 53 61 74 89 66 139 83 66 63  
ybr046c 490 456 591 907 361 685 502 552 550  
ybr047w 59 75 92 171 71 130 82 68 59  
ybr048w 7875 9083 6500 11895 10398 16270 10546 12846 10288  
ybr049c 373 646 400 641 721 1291 637 665 633  
ybr050c 236 189 204 392 184 343 190 270 236  
ybr051w 3 9 13 10 14 33 5 5 14  
ybr052c 1375 1535 1774 2562 1517 2852 1633 2064 1866  
ybr053c 565 555 645 999 407 899 513 728 567  
ybr054w 477 344 204 877 252 709 726 303 345  
ybr055c 199 447 336 433 390 882 387 386 296  
ybr056w 879 960 987 1762 750 1594 1054 1128 958  
ybr056w-a 6 10 7 37 10 12 21 6 14  
ybr057c 376 759 647 740 693 1197 643 625 793  
ybr058c 400 719 481 825 682 1337 719 709 632  
ybr058c-a 340 563 442 607 511 1006 454 440 603  
ybr059c 1413 1386 1253 2060 1442 2970 1634 1958 1560  
ybr060c 216 509 323 454 455 902 403 365 396  
ybr061c 119 274 205 245 292 527 260 231 229  
ybr062c 243 368 315 491 305 559 313 366 401  
ybr063c 58 177 105 149 156 325 103 116 81  
ybr065c 45 102 77 108 105 221 102 89 99  
ybr066c 119 422 237 321 415 684 286 227 342  
ybr067c 3589 7275 5076 6368 7937 14276 6530 7873 8160  
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ydr024w 25 19  
ydr025w 10998 9679  
ydr026c 785 700  
ydr027c 949 665  
ydr028c 2814 2171  
ydr029w 43 24  
ydr030c 141 85  
ydr031w 251 179  
ydr032c 3871 3272  
ydr033w 641 549  
ydr034c 512 404  
ydr034c-a 12 11  
ydr034c-d 488 394  
ydr034w-b 74 63  
ydr035w 3099 2823  
ydr036c 519 394  
ydr037w 6322 5791  
ydr038c 829 828  
ydr039c 928 948  
ydr040c 926 889  
ydr041w 1014 782  
ydr042c 47 30  
ydr043c 309 266  
ydr044w 1061 852  
ydr045c 424 381  
ydr046c 2221 2110  
ydr047w 1633 1512  
ydr049w 659 512  
ydr050c 48313 44357  
ydr051c 796 693  
ydr052c 1239 859  
ydr054c 2558 1595  
ydr055w 749 592  
ydr056c 1530 1278  
ydr057w 781 565  
ydr058c 292 213  
ydr059c 740 604  
ydr060w 982 870  
 [ reached getOption("max.print") -- omitted 5329 rows ]

$names  
[1] "counts" "samples" "genes"   
  
$class  
[1] "DGEList"  
attr(,"package")  
[1] "edgeR"

[1] 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23  
[24] 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46  
[47] 47 48 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21  
[70] 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44  
[93] 45 46 47 48

Framework - : observed count for gene in sample (library) - : number of genes -

: total number of reads for sample - : number of samples in the experiment - : normalization factor associated with sample

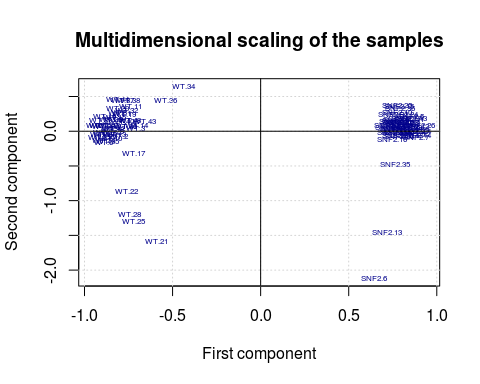
### Total read count normalization

Motivation – scaling to library size as a form of normalization makes intuitive sense, given it is expected that sequencing a sample to half the depth will give, on average, half the number of reads mapping to each gene. Assumption – read counts are proportional to expression level and sequencing depth. Method – divide transcript read count by total number of reads and rescale the factors to counts per million

WT.1 WT.2 WT.3 WT.4 WT.5 WT.6 WT.7 WT.8 WT.9   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06   
 WT.10 WT.11 WT.12 WT.13 WT.14 WT.15 WT.16 WT.17 WT.18   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06   
 WT.19 WT.20 WT.21 WT.22 WT.23 WT.24 WT.25 WT.26 WT.27   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06   
 WT.28 WT.29 WT.30 WT.31 WT.32 WT.33 WT.34 WT.35 WT.36   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06   
 WT.37 WT.38 WT.39 WT.40 WT.41 WT.42 WT.43 WT.44 WT.45   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06   
 WT.46 WT.47 WT.48 SNF2.1 SNF2.2 SNF2.3 SNF2.4 SNF2.5 SNF2.6   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06   
 SNF2.7 SNF2.8 SNF2.9 SNF2.10 SNF2.11 SNF2.12 SNF2.13 SNF2.14 SNF2.15   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06   
SNF2.16 SNF2.17 SNF2.18 SNF2.19 SNF2.20 SNF2.21 SNF2.22 SNF2.23 SNF2.24   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06   
SNF2.25 SNF2.26 SNF2.27 SNF2.28 SNF2.29 SNF2.30 SNF2.31 SNF2.32 SNF2.33   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06   
SNF2.34 SNF2.35 SNF2.36 SNF2.37 SNF2.38 SNF2.39 SNF2.40 SNF2.41 SNF2.42   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06   
SNF2.43 SNF2.44 SNF2.45 SNF2.46 SNF2.47 SNF2.48   
 1e+06 1e+06 1e+06 1e+06 1e+06 1e+06

## Multidimensional scaling

Each sample can be viewed as a vector of 6370 values (one value per gene). We can thus imagine a "sample space" made of 6370 dimensions (one dimension per gene) , where the position of each sample is defined by the normalized counts (CPM) of each gene.



### Comments (JvH)

You have a matrix with 96 samples and 7200 genes. You can consider this table in 2 ways:

1. You are interested by samples -> each sample (column) will correspond to an "object of interest" and each row (gene) will be one variable that characterizes this object.
2. Alternatively, you might be interested by genes (rows) and consider each row as one object of interest (gene) which is characterized by its count values in the 96 samples. In this case, the gene is the "object" and the sample is the variable.

For now on, we will consider that the objects of interest are the samples. Each sample is defined by a vector of 7200 numbers (each column of the count table is a vector), which correspond to 7200 variables (counts per gene).

**Multidimensional scaling** aims at reducing the number of dimensions of a dataset, for various purposes: visualization, variable selection, .... In our case, our 96 samples are characterized by 7200 variables. This is huge ! We have many more variables (genes) than samples. One convenient way to perceive the relationships between samples is to **project** them onto a 2-dimensional space (a plane), for the sake of visualization. This means that you must reduce your 7200 dimensions to no more than 2 dimensions. Various methods exist for multidimensional scaling:

* Principal Component Analysis followed by a selection of the 2 first components.
* **Singular value decomposition** (needs prior computation of a distance matrix between each pair of objects)
* Spring embedding from a distance matrix (needs prior computation of a distance matrix between each pair of objects)

The method plotMDS apparently relies on the SVD method. It does something a bit more complicated, because it computes the distance matrix with a subset of the genes (without actually explaining how this subset is selected), and then applies multidimensional scaling to reduce the dimensions to a user-specified number (by default, they return only 2 dimensions).

**Interpretation of the plotMDS result**: in the two first components, the distance between samples is an approximation of the similarity betwen their count profiles per gene (the vectors corresponding to the columns in your count table).

Interestingly, the first component perfectly separates the WT from the Snf2 samples (the two genotypes). We also notice the presence of some "outliers", i.e. WT samples projecting outside of the WT cluster, and Snf2 samples projected outside of the Snf2 cluster.

Within each genotype (WT or SNF2, resp.) the samples are highly concentrated in the PC1-PC2 plane. However, for each genotype, a very few samples are discading from the other ones. For example, WT.21, WT.25, WT.28, ... WT.34 for the WT genotype, and SNF2.6, SNF2.13, SNF2.35 for the SNF2 genotype). This might reflect problems with these particular samples, and one might consider them as outliers, and discard them for further analysis. This is a choice that will affect the rest of the analysis.

# Setting up the Model

We will specify the design of our Matrix, which describes the setup of our experiment.

sample.type  
SNF2 WT   
 48 48

(Intercept) sample.replicates sample.typeWT  
1 1 1 1  
2 1 2 1  
3 1 3 1  
4 1 4 1  
5 1 5 1  
6 1 6 1  
7 1 7 1  
8 1 8 1  
9 1 9 1  
10 1 10 1  
11 1 11 1  
12 1 12 1  
13 1 13 1  
14 1 14 1  
15 1 15 1  
16 1 16 1  
17 1 17 1  
18 1 18 1  
19 1 19 1  
20 1 20 1  
21 1 21 1  
22 1 22 1  
23 1 23 1  
24 1 24 1  
25 1 25 1  
26 1 26 1  
27 1 27 1  
28 1 28 1  
29 1 29 1  
30 1 30 1  
31 1 31 1  
32 1 32 1  
33 1 33 1  
34 1 34 1  
35 1 35 1  
36 1 36 1  
37 1 37 1  
38 1 38 1  
39 1 39 1  
40 1 40 1  
41 1 41 1  
42 1 42 1  
43 1 43 1  
44 1 44 1  
45 1 45 1  
46 1 46 1  
47 1 47 1  
48 1 48 1  
49 1 1 0  
50 1 2 0  
51 1 3 0  
52 1 4 0  
53 1 5 0  
54 1 6 0  
55 1 7 0  
56 1 8 0  
57 1 9 0  
58 1 10 0  
59 1 11 0  
60 1 12 0  
61 1 13 0  
62 1 14 0  
63 1 15 0  
64 1 16 0  
65 1 17 0  
66 1 18 0  
67 1 19 0  
68 1 20 0  
69 1 21 0  
70 1 22 0  
71 1 23 0  
72 1 24 0  
73 1 25 0  
74 1 26 0  
75 1 27 0  
76 1 28 0  
77 1 29 0  
78 1 30 0  
79 1 31 0  
80 1 32 0  
81 1 33 0  
82 1 34 0  
83 1 35 0  
84 1 36 0  
85 1 37 0  
86 1 38 0  
87 1 39 0  
88 1 40 0  
89 1 41 0  
90 1 42 0  
91 1 43 0  
92 1 44 0  
93 1 45 0  
94 1 46 0  
95 1 47 0  
96 1 48 0  
attr(,"assign")  
[1] 0 1 2  
attr(,"contrasts")  
attr(,"contrasts")$sample.type  
[1] "contr.treatment"

# Estimating Dispersions

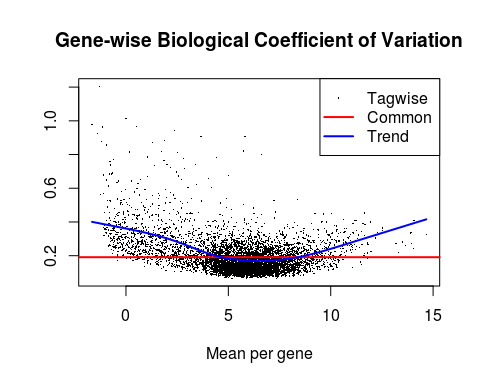
Now, we will estimate the dispersion parameter for our negative Bionomial Model. owing to it is difficult to estimate the dispersion accurately for each Gene. therefore we will take a way of **sharing information between genes** BY

* using a common estimate across all genes.
* Fitting an estimate based on the Mean-Variance trend across the dataset, such that genes similar abundances have similar variance estimates (Trended dispersion)
* Computing a genewise dispersion (tagwise dispersion).

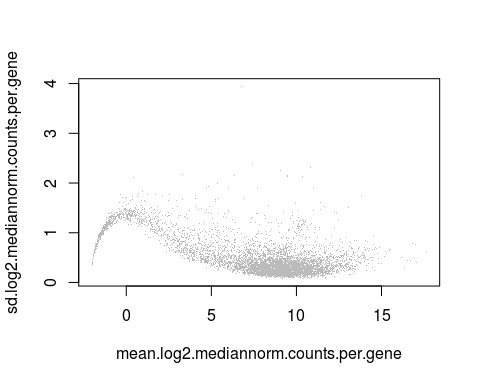
In edgeR, We use an empirical Bayes method to "Shrink" the genewise dispersion estimates towards the common dispersion (tagwise dispersion). we must to estimate common or trended dispersion before we can estimate the tagwise dispersion.

Disp = 0.03662 , BCV = 0.1914

Disp = 0.14661 , BCV = 0.3829   
Disp = 0.10692 , BCV = 0.327   
Disp = 0.10005 , BCV = 0.3163   
Disp = 0.08874 , BCV = 0.2979   
Disp = 0.06871 , BCV = 0.2621   
Disp = 0.05391 , BCV = 0.2322   
Disp = 0.0444 , BCV = 0.2107   
Disp = 0.03938 , BCV = 0.1985   
Disp = 0.03555 , BCV = 0.1885   
Disp = 0.03552 , BCV = 0.1885   
Disp = 0.03407 , BCV = 0.1846   
Disp = 0.0317 , BCV = 0.178   
Disp = 0.02768 , BCV = 0.1664   
Disp = 0.03061 , BCV = 0.1749   
Disp = 0.0269 , BCV = 0.164   
Disp = 0.0335 , BCV = 0.183   
Disp = 0.0336 , BCV = 0.1833   
Disp = 0.02743 , BCV = 0.1656   
Disp = 0.0287 , BCV = 0.1694   
Disp = 0.0299 , BCV = 0.1729   
Disp = 0.02785 , BCV = 0.1669   
Disp = 0.03338 , BCV = 0.1827   
Disp = 0.03162 , BCV = 0.1778   
Disp = 0.02875 , BCV = 0.1696   
Disp = 0.02775 , BCV = 0.1666   
Disp = 0.02896 , BCV = 0.1702   
Disp = 0.03132 , BCV = 0.177   
Disp = 0.0296 , BCV = 0.172   
Disp = 0.03168 , BCV = 0.178   
Disp = 0.03307 , BCV = 0.1818   
Disp = 0.03735 , BCV = 0.1933   
Disp = 0.04496 , BCV = 0.212   
Disp = 0.07021 , BCV = 0.265



### Manual exploration of the method



Notes:

* since some genes may have zero counts in several samples, the corresponding counts were replaced by an arbitrary epsilon, smaller than 1 -> the log2 is smaller than 0. Consequently, the mean log2-counts can be negative for some genes

# Differential Expression

Now, we can find our differentially expressed genes. After fitting the Model, we can use **topTags()** function to explore the results, and set thresholds to identify subset of differentially expressed genes.

$class  
[1] "TopTags"  
attr(,"package")  
[1] "edgeR"  
  
$names  
[1] "table" "adjust.method" "comparison" "test"

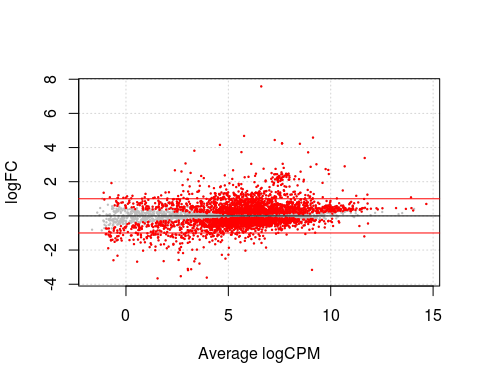
genes logFC logCPM LR PValue FDR  
6105 yor290c 7.581310 6.606534 10382.777 0 0  
2970 yhr136c 4.682153 5.768156 2107.559 0 0  
4802 yml123c 4.583783 9.134681 5164.289 0 0  
3067 yhr215w 4.443164 7.266128 6317.476 0 0  
1789 yer011w 4.248580 7.625934 2898.275 0 0  
179 yar071w 4.242678 7.621323 6497.114 0 0  
2682 ygr234w 4.221224 8.494908 6824.282 0 0  
1399 ydr281c 3.815549 3.340019 2283.355 0 0  
2466 ygr035c 3.735650 5.639477 1656.123 0 0  
1127 ydr033w 3.721760 8.897485 3129.047 0 0

deGenes  
 -1 0 1   
1943 2545 1882

[1] 6370

[1] 3825

[1] 0.600471



### Interpretation

The edgeR::plotSmear() method draws n equivalent of the "MA plot'" traditionally used for microarrays.

Mustafa: find some doc on the MA plot interpretation, for example <https://en.wikipedia.org/wiki/MA_plot>

In short:

* each dot corresponds to one gene;
* the abcissa indicates the average (A) level of expression for the two conditions;
* the ordinate indicates the log-ratio of expression.
  + Positive values indicate **up-regulation**, i.e. that the gene is expressed at a higher level in mutant (Snf2) than in wild-type
  + Negative values indicate **down-regulation**: the gene is expressed at a lower level in the mutant than in the wild-type.
  + the red dots highlight the genes declared significant by edgeR

Note: the smear plot shows strange results: a lot of genes are declared significantly differentially expressed (~3800, i.e. 60% of all genes). This is a huge number of genes, but the plot shows that many of them have a relatively small logFC, which indicates that the mean expression values are quite close between WT and Snf2.

How to interpret this ? - check if the genes declared significant with a small logFG correspond to genes with a very small variance.

# Estimating Dispersions

The first dispersion type to calculate is the common dispersion. **in the common dispersion stting** each gene gets assigned the same dispersion estimate. The output of the estimation will include the estimate as well as some other elements added to the edgeR Object, cds.

[1] "counts" "samples" "common.dispersion"  
[4] "pseudo.counts" "pseudo.lib.size" "AveLogCPM"

[1] 0.09154957

[1] 75.04295

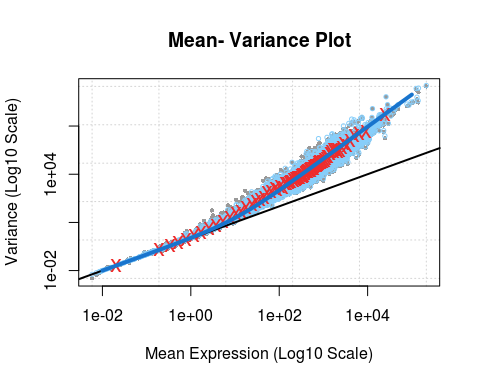
Min. 1st Qu. Median Mean 3rd Qu. Max.   
0.003338 0.039930 0.067150 0.118200 0.131400 5.859000

Min. 1st Qu. Median Mean 3rd Qu. Max.   
0.003997 0.041250 0.068540 0.116600 0.133600 3.840000

"to undesstand what this value means, recall the parameterization for the variance of the negative Bionomial is

. for Poisson it's

. the implied Standard Deviations are the squere-roots of the variances. Once the common disprsion is estimated we can estimate the tagwise dispersions. in our senario, each gene will get its own unique dispersion estimate. but the common dispersion is still used in the calcuation. The tagwise disparsions are squeezed toward the common value.\*\* the amount of squeezing\*\* is governed by the paramter prior.n (italic). The higher prior.n, the closer the estimates will be to the common dispersion. The recommended value is the nearest integer to 50/(#samples - #groups). For our yeast dataset that's round(50/(96-2))=1" Quoted from S.Ruddy, 2011.



CountDataSet (storageMode: environment)  
assayData: 7125 features, 96 samples   
 element names: counts   
protocolData: none  
phenoData  
 sampleNames: WT.1 WT.2 ... SNF2.48 (96 total)  
 varLabels: sizeFactor condition  
 varMetadata: labelDescription  
featureData: none  
experimentData: use 'experimentData(object)'  
Annotation:

WT.1 WT.2 WT.3 WT.4 WT.5 WT.6 WT.7   
0.5857207 0.9246561 0.7507708 1.1092268 0.8830085 1.6867285 0.8962644   
 WT.8 WT.9 WT.10 WT.11 WT.12 WT.13 WT.14   
0.9399462 0.8892189 0.9107280 0.6425402 0.8357242 1.0222096 1.0997359   
 WT.15 WT.16 WT.17 WT.18 WT.19 WT.20 WT.21   
0.7815938 0.5501050 0.8853992 0.8779457 0.9386570 0.6311914 1.0525968   
 WT.22 WT.23 WT.24 WT.25 WT.26 WT.27 WT.28   
1.4439790 0.8696882 0.9096221 1.1941166 0.9740209 1.0486777 1.1778201   
 WT.29 WT.30 WT.31 WT.32 WT.33 WT.34 WT.35   
0.8766584 1.2581823 0.9392171 0.6437706 0.9773164 0.8280041 0.7687677   
 WT.36 WT.37 WT.38 WT.39 WT.40 WT.41 WT.42   
0.7806107 0.9222572 0.6465259 0.7142891 1.2771748 1.0632859 0.8032591   
 WT.43 WT.44 WT.45 WT.46 WT.47 WT.48 SNF2.1   
0.7389226 0.6908594 1.1660750 0.6040710 1.2718789 0.8539195 1.3945693   
 SNF2.2 SNF2.3 SNF2.4 SNF2.5 SNF2.6 SNF2.7 SNF2.8   
1.0855205 1.0784430 1.4473009 0.8746661 1.3742122 1.7170980 0.9669422   
 SNF2.9 SNF2.10 SNF2.11 SNF2.12 SNF2.13 SNF2.14 SNF2.15   
1.2698050 0.9765104 1.1859748 0.9695162 1.2534427 1.2779649 0.9057394   
 SNF2.16 SNF2.17 SNF2.18 SNF2.19 SNF2.20 SNF2.21 SNF2.22   
1.2336380 1.2753405 1.1178957 0.9172253 1.1242619 1.1986067 0.8699097   
 SNF2.23 SNF2.24 SNF2.25 SNF2.26 SNF2.27 SNF2.28 SNF2.29   
0.9967392 1.6737040 0.9105913 1.3123592 0.8222665 0.9664385 1.0762787   
 SNF2.30 SNF2.31 SNF2.32 SNF2.33 SNF2.34 SNF2.35 SNF2.36   
0.9367890 0.8585266 1.2654411 1.5593815 1.1333540 0.9528620 0.9741423   
 SNF2.37 SNF2.38 SNF2.39 SNF2.40 SNF2.41 SNF2.42 SNF2.43   
1.0779583 0.9880958 1.9583925 1.0734732 1.2251204 0.8501760 1.2209626   
 SNF2.44 SNF2.45 SNF2.46 SNF2.47 SNF2.48   
1.2570805 0.9888757 1.1460598 1.2289630 0.9761410

WT.1 WT.2 WT.3 WT.4 WT.5 WT.6  
21s\_rrna 34.145969 82.192720 134.528414 89.251357 144.958975 43.871910  
hra1 5.121895 2.162966 2.663929 1.803058 3.397476 2.964318  
icr1 128.047383 133.022429 142.520201 141.540031 110.984215 145.251593  
lsr1 102.437906 176.281755 310.347728 146.949204 218.570954 222.323867  
nme1 22.194880 15.140764 30.635183 11.719875 32.842268 23.714546  
pwr1 0.000000 0.000000 0.000000 0.000000 0.000000 0.000000  
 WT.7 WT.8 WT.9 WT.10 WT.11 WT.12  
21s\_rrna 40.166721 34.04450 64.10120 114.194360 301.92665 13.162237  
hra1 4.462969 0.00000 4.49833 4.392091 0.00000 7.179402  
icr1 132.773326 127.66688 133.82531 144.938995 141.62539 117.263565  
lsr1 216.453994 89.36681 237.28690 173.487585 101.16099 165.126244  
nme1 31.240783 24.46948 29.23914 24.156499 14.00691 13.162237  
pwr1 0.000000 0.00000 0.00000 0.000000 0.00000 0.000000  
 WT.13 WT.14 WT.15 WT.16 WT.17 WT.18  
21s\_rrna 39.130918 68.198192 25.58874 401.74150 134.402648 307.5361  
hra1 1.956546 3.637237 0.00000 0.00000 6.776604 0.0000  
icr1 123.262391 141.852240 145.85581 127.24844 145.696989 152.6290  
lsr1 194.676315 119.119510 126.66425 570.80014 232.663408 468.1383  
nme1 22.500278 8.183783 19.19155 59.98855 24.847548 50.1170  
pwr1 0.000000 0.000000 0.00000 0.00000 0.000000 0.0000  
 WT.19 WT.20 WT.21 WT.22 WT.23 WT.24  
21s\_rrna 94.816316 413.503748 25.650849 109.419870 125.332270 50.570451  
hra1 3.196056 3.168611 4.750157 2.077592 4.599349 5.496788  
icr1 125.711520 141.003194 150.104967 136.428572 132.231294 127.525485  
lsr1 123.580816 736.702081 184.306098 293.633069 259.863238 152.810710  
nme1 14.914926 50.697778 11.400377 25.623640 43.693819 26.384583  
pwr1 0.000000 0.000000 0.000000 0.000000 0.000000 0.000000  
 WT.25 WT.26 WT.27 WT.28 WT.29  
21s\_rrna 52.758668 361.388548 51.4934193 48.3944873 35.36155  
hra1 1.674878 2.053344 0.9535818 0.8490261 4.56278  
icr1 116.404046 113.960593 126.8263845 121.4107313 118.63229  
lsr1 231.970653 411.695477 191.6699495 177.4464534 174.52635  
nme1 20.098540 37.986864 23.8395460 19.5276001 20.53251  
pwr1 0.000000 0.000000 0.0000000 0.0000000 0.00000  
 WT.30 WT.31 WT.32 WT.33 WT.34 WT.35  
21s\_rrna 48.482642 36.200364 118.054483 112.55311 121.980067 111.867349  
hra1 2.384392 7.453016 1.553348 5.11605 4.830894 2.601566  
icr1 147.832319 141.607305 128.927923 114.59953 103.864216 123.574397  
lsr1 140.679142 282.149893 124.267877 270.12746 317.631264 290.074638  
nme1 17.485543 36.200364 29.513621 45.02124 31.400809 26.015663  
pwr1 0.000000 0.000000 0.000000 0.00000 0.000000 0.000000  
 WT.36 WT.37 WT.38 WT.39 WT.40 WT.41  
21s\_rrna 247.24233 31.444589 109.817728 13.999933 214.536024 46.083559  
hra1 0.00000 4.337185 1.546729 6.999966 2.348935 5.642885  
icr1 108.88911 120.356877 98.990628 118.999428 138.587140 128.845870  
lsr1 285.67378 108.429618 85.070071 92.399556 301.446604 218.191547  
nme1 28.18306 21.685924 20.107471 8.399960 40.714866 29.154905  
pwr1 0.00000 0.000000 0.000000 0.000000 0.000000 0.000000  
 WT.42 WT.43 WT.44 WT.45 WT.46 WT.47  
21s\_rrna 37.347850 97.439159 83.953409 18.009133 263.214071 84.127506  
hra1 6.224642 2.706643 2.894945 1.715155 8.277172 1.572477  
icr1 146.901543 152.925346 117.245278 121.776040 155.610834 147.026576  
lsr1 185.494321 154.278668 117.245278 93.475975 218.517342 191.055924  
nme1 27.388423 13.533216 21.712088 19.724288 16.554344 31.449535  
pwr1 0.000000 0.000000 0.000000 0.000000 0.000000 0.000000  
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## Estimation of variance

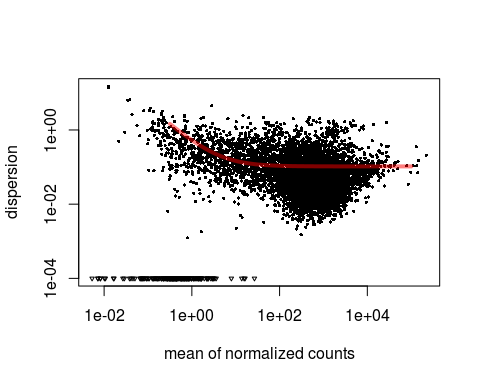
in DESeq, the inference relies on the estimation of the typical relationship between the data's viaraince (the data dispersion) and their mean. where is \*\* the dispersion\*\* can understood as the square of the coefficient of Biological variation, so, if a gene's expression typically differs from replicate to replicate sample by 20%thence gene's dispersion is 0.2 power 2 = 0.04. we can Note the Variance between counts is the sum of two components: the sample-to-sample variation and uncertinity in measuring a concentration by counting reads. this emply us to know shot nois or poisson noise, which is the dominating noise source for lowly expressed genes. The former dominates for highly expressed genes is the sum of both, shot noise and dispersion, is considered in the differential expression inference. of count vaue is modelled as

**the variance**

,

* where is expected normalized count value (estimated by the average normalised count value).
* is the sive factor for the sample under considaration.
* is the dispertion value for the gene under consideration.

List of 5  
 $ perGeneDispEsts: num [1:7125] 1.4415 0.2451 0.0155 0.4596 0.2704 ...  
 $ dispFunc :function (q)   
 ..- attr(\*, "coefficients")= Named num [1:2] 0.104 0.428  
 .. ..- attr(\*, "names")= chr [1:2] "asymptDisp" "extraPois"  
 ..- attr(\*, "fitType")= chr "parametric"  
 $ fittedDispEsts : num [1:7125] 0.108 0.274 0.107 0.106 0.12 ...  
 $ df : int 48  
 $ sharingMode : chr "maximum"



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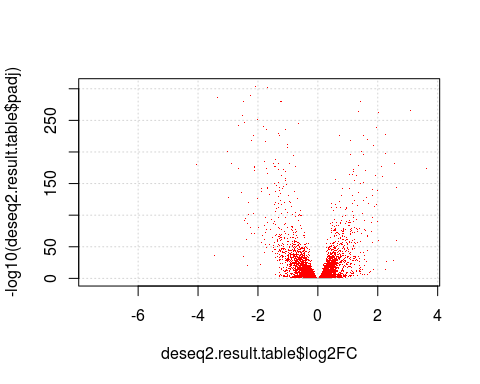
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[7123] 4.0710074 3.8708909 Inf

# inference: by calling Differential expression

# Differential analysis with DESeq2

out of 6886 with nonzero total read count  
adjusted p-value < 0.1  
LFC > 0 (up) : 2613, 38%   
LFC < 0 (down) : 2522, 37%   
outliers [1] : 0, 0%   
low counts [2] : 0, 0%   
(mean count < 0)  
[1] see 'cooksCutoff' argument of ?results  
[2] see 'independentFiltering' argument of ?results

FALSE TRUE   
 2027 4859



For next time (start inquiring on your site):

* multiple testing corrections, including
  + nominal p-value
  + False Positive Rate versus False Discovery Rate: understand the difference
  + Bonferroni
  + Benjamini-Hochberg

Next time we discuss about the interpretation of the differentially expressed genes with DESeq2.

# Estimation of variance

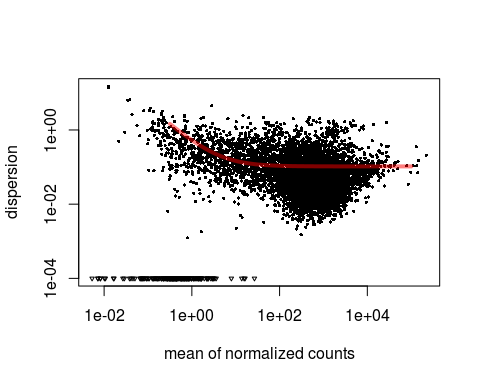
In DESeq, the inference relies on the estimation of the typical relationship between the data's viaraince (the data dispersion) and their mean. where is \*\* the dispersion\*\* can understood as the square of the coefficient of Biological variation, so, if a gene's expression typically differs from replicate to replicate sample by 20%thence gene's dispersion is 0.2 power 2 = 0.04. we can Note the Variance between counts is the sum of two components: the sample-to-sample variation and uncertinity in measuring a concentration by counting reads. this emply us to know shot nois or poisson noise, which is the dominating noise source for lowly expressed genes. The former dominates for highly expressed genes is the sum of both, shot noise and dispersion, is considered in the differential expression inference.$$v of count vaue is modelled as

\*\*\*\*\* the variance

$$v = \sm \sum $ m$$

( column), \* where M is expected normalized count value (estimated by the average normalised count value). \* s is the sive factor for the sample under considaration. \* is the dispertion value for the gene under consideration.

List of 5  
 $ perGeneDispEsts: num [1:7125] 1.4415 0.2451 0.0155 0.4596 0.2704 ...  
 $ dispFunc :function (q)   
 ..- attr(\*, "coefficients")= Named num [1:2] 0.104 0.428  
 .. ..- attr(\*, "names")= chr [1:2] "asymptDisp" "extraPois"  
 ..- attr(\*, "fitType")= chr "parametric"  
 $ fittedDispEsts : num [1:7125] 0.108 0.274 0.107 0.106 0.12 ...  
 $ df : int 48  
 $ sharingMode : chr "maximum"



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1. Rueda and Pereira (2015) [↑](#footnote-ref-76)