

Figure 1

Run quality check tools

Choose the quality checks you want to include in your analysis and click Next to continue.

If you don't want any quality checking you can skip this step.

Info

Boxplots and MA plots
The boxplots visualize the distribution of unprocessed log scale probe intensities. Generally in a perfect experiment all the plots should be in agreement with each other. Chips that show a strongly deviating probe intensity distribution might be problematic.

In the MA plots, the normalized log scale probe intensities are plotted against the log fold change in expression when compared to a synthetic chip that was created by taking probe-wise medians of all chips in the input set. A loess curve is fitted to the scatter plot and the deviation of the data from the theoretically expected zero-line is estimated via the integral of the loess curve over the zero-line. In addition to this the amount of probes that show a more than 2 fold change in expression are recorded and a warning is issued if the amount exceeds 5% of the genes.

Box plot and MA plots ☐ Include? [more](#)

PLM – Fitting probe level models to the data ☐ Include? [more](#)

RNA digestion – Uses ordered probes in probeset to detect possible RNA degradation ☐ Include? [more](#)

Histogram – Shows density plots of the signal intensity for each chip ☐ Include? [more](#)

Scatterplot – The data will be normalised before plotting the log2-fold expression values of all possible combinations of two chips against each other ☐ Include? [more](#)

PCA and hierarchical clustering ☐ Include? [more](#)

NUSE and RLE – Normalized unscaled standard errors and relative logarithmic expression ☐ Include? [more](#)

☒ Edit expert options
The chosen default values will be suitable in most cases. They should only be changed by an expert.

Select all ☐

Expert settings

Normalisation method [?](#)

p value correction method [?](#)

Analysis strategy [?](#)

Design your experiment

You can arrange the groups by dragging them around. Define which groups shall be compared by holding down the **CONTROL** key and then click-dragging from the **first group** to the **second group**. Right-click and choose delete to delete connections.

To combine several groups into one "metagroup" select all groups you want to combine (by left-clicking and drawing a selection rectangle around them) and click "Create Metagroup".

☒ Show expert settings

Expert settings

Normalisation:

P value correction:

Multiple testing strategy:

☒ Write out normalized raw data

☐ Preview R script

☒ Log-fold change min=1

p-value cutoff:

```

graph TD
    WT[wildtype] --> WTst[wildtype stressed]
    M[mutant] --> Mst[mutant stressed]
    WTst --> WTstMst[wildtype-wildtype stressed]
    Mst --> MstMst[mutant-mutant stressed]
    WTstMst --> MstMst
        
```

Figure 1: (A) Screenshot of the quality assessment functions available for Affymetrix (R) chips. All methods can be freely combined to obtain an overview of the input data quality. Short inline explanations for each method are displayed in the info field on the left side upon clicking the question marks. The expert panel at the bottom of the user interface is providing more option for customizing the analysis settings. By default, robust analysis methods are predefined and panel is hidden to provide a less cluttered interface to inexperienced users. (B) Screenshot of the graphical experiment designer panel. Comparisons between the previously defined groups of biological replicate chips can be configured by dragging visual connections between them. The arrowhead defines the direction of the comparison. E.g. the arrow between the 'wildtype' group and the 'wildtype stress' group is interpreted as the 'wildtype - wildtype stress' contrast, meaning that genes showing a higher expression level in the 'wildtype stress' group will have a negative log2 fold change value in the output and vice versa. Interaction terms can be defined via 'metagroups' shown as orange boxes.